SECTION 2

ZANTHOXYLUM RHETSA
PHYTOCHEMICAL INVESTIGATION OF STEM BARK PRICKLES
1. THE GENUS ZANTHOXYLUM

The family Rutaceae consists of 150 genera and nearly 900 species, many of which are sources of essential oils used in perfumery and medicine. Among the plants belonging to this family, Zanthoxylum is the largest and most widespread and are native to warm temperate and subtropical areas. In Asia, this genus is often found in the Himalayan region and also in Central, South, Southeast and East Asia. The different species of this genus demonstrate very close relationship among them and are identified as trees, erect shrubs or small trees, straggling or scandent shrubs or as a forest liane, most of which are aromatic with spiny trunks and branches. A common feature of most of the species of this genus is the wonderful capacity to produce gums and volatile oils. The fruits of Zanthoxylum and their pericarps are used as a peppery spice in both sweet and savory preparations and the seeds rich in oil are often used as fertilizer or fuel. The seeds are also used in folk medicine for the treatment of stomachache, toothache, abdominal pain, ascariosis, diarrhoea and dysentery. It is also used to promote digestion and as a topical antibacterial agent for treatment of infected wounds. The essential oils from the fruits have shown anthelmintic effects. Traditional healers have used different species of the Zanthoxylum for treatment of a wide range of disorders, including urinary and venereal diseases, rheumatism and lumbago.

Zanthoxylum is represented by around 12 species from India which are Z. acanthopodium, Z.armatum, Z.ovalifolium, Z.tomentellum, Z.rhetsa, Z.myriacanthum, Z.pseudoxyphyllum, Z.tetraspermum, Z.burkillianum, Z.nitidum, Z.scandens and Z.oxyphyllum. Chemical studies carried out on Zanthoxylum species have revealed the occurrence mainly of alkaloids, aliphatic and aromatic amides, lignans, coumarins, sterols, carbohydrate residues etc. Some of these metabolites have reported cytotoxic,
molluscicidal, anticonvulsant, anti-sickling, anesthetic, antibacterial, anti-hypertensive and anti-inflammatory properties. The alkaloids commonly found in species of the genus *Zanthoxylum* belong to groups of isoquinoline (mainly benzo[c]phenanthridine-type) and quinoline and can be demonstrated as models for anti tumor drugs. The chemical diversity of the Rutaceous alkaloids is correlated with biosynthetic pathways involving various amino acid precursors\(^3\). Apart from all the studies carried out, science has not lost interest in this genus of plants yet because it can be understood from the reports on them that there is much more to be investigated. There are recent reports of novel compounds of very interesting structural features and also compounds possessing obvious biological properties from *Zanthoxylum* as is given below.

*Zanthoxylum ailanthoides*

*Z. ailanthoides* Sieb. & Zucc. is a medium to large sized tree found at low altitude in forests of China, Korea, Japan, Philippines, and Taiwan. Its leaves are used as a folk medicine to treat common cold. Benzo[c]phenanthridines, quinolines, coumarins, flavonoids, lignans, and terpenoids have been identified as constituents of this plant. Antiplatelet aggregation and anti-HIV activities have been reported for some of the isolated compounds\(^4\). The root, stem, leaves and fruits of *Z. ailanthoides* are reported to have several health benefits, such as myocardium disorder attenuation, bone-injury alleviation and cold resistance\(^5\). In China, the bark has been prescribed for rheumatism, arthralgia, stasis, contusions, and snakebite, and to stimulate blood circulation.

The root bark of the plant has shown anti-HIV activity in vitro and two components, xanthyletin and hesperidin have been isolated from it. On evaluating the inhibition of HIV replication in H9 lymphocyte cells for sixty seven compounds obtained from the root bark of *Z. ailanthoides*, 14
compounds demonstrated significant activity, among which decarine, γ-fagarine, and (+)-tembamide were the most potent anti-HIV compounds, with EC$_{50}$ values of <0.1µg/mL and TI values of >226, >231, and >215, respectively.$^6$

It is reported that flavonoids including rutin and hyperoside from the leaves of Z. ailanthoides have the protective effect on LDL oxidation and lipid accumulation in macrophage. The chloroform soluble extracts of leaves of Z. ailanthoides showed cytotoxic activity against human promyelocytic leukemia (HL-60) and myelomonocytic leukemia (WEHI-3) cells with IC$_{50}$ values of 73.06 and 42.22g/mL respectively. Four compounds, pheophorbide-a methyl ester, pheophorbide-b methyl ester, 13-hydroxyl (13-S) pheophorbide-a methyl ester and 13-hydroxyl (13-R) pheophorbide-b methyl ester, were isolated from this fraction. Except the first, all other three compounds showed cytotoxic activities against both leukemia cells with IC$_{50}$ value in the range of 46.76–79.43µM, whereas first compound exhibited only weak cytotoxic activity. The compounds also induced apoptosis and DNA damage in leukemia cells after treatment. These results suggested that the Z. ailanthoides is biologically active against leukemia cells.$^7$

It is known that the oxidation of low-density lipoprotein play a key role in determining the fate of lesion formation and progression, in the case of atherosclerosis. In addition, the oxisidised form of the lipoprotein is readily taken up by macrophages that mediate endocytosis which also contributes significantly to atherosclerotic lesion formation.$^8$ Therefore, reducing the process of such an oxidation and lipoprotein uptake would be a crucial consideration of protection against atherogenesis. Chu and coworkers explored the effect of fresh leaf extract of Z. ailanthoides on the oxidation of low-density lipoprotein by employing copper (II) sulfate (CuSO$_4$) as an oxidative inducer. Their data showed that the extract reduced the oxidation
properties of the lipoprotein induced by CuSO₄. In addition, it inhibited lipid accumulation in differentiated THP-1 cells treated with the oxidized form involving decreasing the expression of scavenger receptors such as scavenger receptor class AI and CD36, which belongs to the class B scavenger receptor. These results demonstrate the protective effect of Z. ailanthoides against the above diseased condition⁹.

The studies on constituents of Formosan plants for in vitro inhibitory activity on neutrophil pro-inflammatory responses revealed Z. ailanthoides to be an active species. The isolation and characterization of three new amides, ailanthamide, N-(4-methoxyphenethyl)-N-methylbenzamide, and (2E,4E)-N-isobutyl-6-oxohepta-2,4-dienamide, two new benzenoids, 4-(4’-hydroxy-3’-methylbutoxy) benzaldehyde and (E)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate and 17 known compounds from the stem bark of Z. ailanthoides and the results regarding the inhibitory activities of all isolates on superoxide generation and elastase release by neutrophils have been communicated in 2009 by Chen and coworkers. The compounds ailanthamide, (2E,4E)-N-isobutyl-6-oxohepta-2,4-dienamide, xanthyletin, decarine, (+)-episesamin, (-)-hinokinin, and evofolin-B were found to exhibit inhibition (IC₅₀ ≤ 5.34 μg/mL) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds ailanthamide, xanthyletin, decarine, and (+)-episesamin also inhibited fMLP/CB-induced elastase release with IC₅₀ values ≤ 5.53μg/mL⁴.

The results of an experiment aimed to evaluate the antioxidative activity and mutagenic properties of various extracts from Z. ailanthoides, showed that all tested extracts had antioxidative characteristics, including the abilities of radical scavenging, reducing, and lipid peroxidation inhibition. It was found that the antioxidative activities of all the extracts increased with the
increase of their concentrations. Ames test was conducted for all the extracts in this study and no mutagenicity effect toward the tester strains (Salmonella typhimurium TA97, TA98, TA100, TA102, and TA1535) was found in any of them. The results suggested that the Z. ailanthoides extracts were safe in genotoxicity and were antioxidants.

**Zanthoxylum alatum**

Z. alatum Roxb syn. Zanthoxylum armatum DC. is an armed, scandent or erect shrub or a small tree (6 m tall or more), with dense foliage, found in the hot valleys of the Himalaya. It is extensively used in the Indian system of medicine as stomachic, carminative and anthelmintic agents. The bark is pungent and is used for preventing toothache. The fruits and seeds are utilized as an aromatic tonic in fever, dyspepsia, cholera and in expelling roundworms. The seeds have deodorant, disinfectant, and antiseptic properties and are used to relieve toothache and their lotions used to cure scabies. A volatile oil consisting mainly linalool, trans-cinnamic acid, nevadensin, umbelliferone, β-sitosterol and its glycoside, tambulin and tambuletin, and unsaturated fatty acids have been reported from the seeds which possess antibacterial, antifungal, anthelmintic, antidiabetic, antiproliferative and insecticidal properties. The plant is also cultivated in some places in China and is used as an analgesic and anti-inflammatory drug in Chinese folk medicine.

The outcome of an investigation to demonstrate the efficacy of Z. alatum seed essential oil as antimicrobial in preservation of food systems and its efficacy as an antioxidant and an antifungal agent revealed a comparable activity of the oil with different synthetic organic preservatives currently used. Also the higher LD$_{50}$ value of the oil versus certain well known botanicals as well as some commercial fungicides indicates its favorable safety profile and negligible mammalian toxicity. The significant antioxidant
activity of the oil may be due the synergism between its components, as neither of the two major components (methyl cinnamate and linalool) individually showed significant free radical scavenging activity. This is a positive indication that the oil may control the development of resistant microorganisms which can occur with organic acid preservatives\textsuperscript{12}.

Chemical studies on the seeds of \textit{Z. alatum} by Akhtara and coworkers led to the isolation of two new phenolic constituents, diphenylalatunoic acid dimethyl ester and zanthoxylum flavone xyloside, along with the five known compounds, 1-methoxy-1,6,3-anthraquinone, 1-hydroxy-6,13-anthraquinone, salicylic acid, methoxysalicylic acid and \(\beta\)-sitosterol-\(\beta\)-D-glucoside\textsuperscript{10}.

A new amide designated as armatamide along with two lignans, asarinin and fargesin, \(\alpha\)-and \(\beta\)-amyrins, lupeol, and \(\beta\)-sitosterol-\(\beta\)-D-glucoside has been isolated from the bark of \textit{Z. alatum} by Kalia and coworkers which is the first report of the presence of a \textit{trans}\textsuperscript{13}cinnamoylamide in the plant.

A lignan named armatumin, along with 25 known compounds was reported from \textit{Z. alatum} and this is the first communication regarding the presence of the isoquinoline alkaloids, norchelerythrine and isodecalone, 4-hydroxybenzoic acid and the furofuran lignans, methylxanthoxylol, kobusin, piperitol and pinoresinol-3,3-dimethylallyl ether in this species. Also the compounds 5’-demethoxyepiexcelsin and epiaschantin were isolated for the first time from Rutaceae in this experiment\textsuperscript{10}.

Allelopathy arises from the release of chemicals by one plant species, which may affect another species growing in its vicinity. Allelopathic influence of \textit{Z. alatum} was tested on some important winter field crops (\textit{T. aestivum, H. vulgare, B. comasris, and L. culminaris}) of Garhwal in the Himalaya region, using different concentrations of leaf, bark, and fruit pulp
aqueous extracts and significant reduction in germination and growth of the test crops were observed\textsuperscript{14}.

A study conducted by Gilani and coworkers describes the gut, airways and cardiovascular modulatory activities of \textit{Z. alatum} which rationalize some of its medicinal uses. Their results indicate that \textit{Z. alatum} exhibits spasmolytic effects, mediated possibly through Ca++ antagonist mechanism, which provides pharmacological base for its medicinal use in the gastrointestinal, respiratory and cardiovascular disorders\textsuperscript{15}. Also when the extracts obtained from four different Nepalese \textit{Zanthoxylum} species were screened for their antiproliferative activity against the growth of human keratinocytes (HaCaT cells), it was found that the extract obtained from \textit{Z. alatum} barks was highly active with an IC\textsubscript{50} value of 11µg/mL\textsuperscript{16}.

\textit{Zanthoxylum ovalifolium}

\textit{Z. ovalifolium} Wight is one of the most widespread species, occurring in mainland Asia and the Malaysian floristic region and in the “wet tropics” bioregion of north-eastern Queensland. From the aerial part of \textit{Z. ovalifolium}, two novel benzo[c]phenanthridine alkaloids, terihanine and isoterihanine were isolated along with nитidine, the unusual furoquinoline 5-methoxydictamine, canthin-6-one and several common furocoumarins. This finding confirms the homogeneity of these species throughout its range from India to Australia. There are two previous studies reported on Australasian material attributable to this species, studied under the names \textit{Z. suberosum} and \textit{Z. dominianum}. These yielded a series of coumarin metabolites and the alkaloid canthin-6-one. Coumarins and canthin-6-one have also been recorded from Indian material together with the furoquinoline alkaloid skimmianine, the benzo[c]phenanthridine norchelerythrine and the indoloquinolizidine rutaecarpine\textsuperscript{17}.
**Zanthoxylum rhoifolium**

*Z. rhoifolium* Lam. is a medium to large tree with dense, sharp prickles on trunk and branches and is used in Amazonia for the treatment of skin eruptions, venereal diseases, and malaria. The leaf essential oil from *Z. rhoifolium* has shown antibacterial effects. The essential oil from the fresh leaves was analysed and a total of 58 compounds were identified accounting for 95.9% of the total oil. It was found to be made up largely of sesquiterpene hydrocarbons (42.9%), monoterpane hydrocarbons (23.1%), and fatty-acid-derived compounds (23.6%), with smaller amounts of oxygenated monoterpenoids and sesquiterpenoids (5.3% and 4.4%, respectively) and aromatic compounds (0.4%). The most abundant essential oil components in *Z. rhoifolium* were germacrene D (14.6%), limonene (12.5%), trans-2-hexenal (11.3%), β-elemene (9.2%), 2-undecanone (9.2%), myrcene (7.9%), bicyclogermacrene (7.5%), and germacrene A (5.2%).

The tertiary benzophenanthridine alkaloid zanthoxyline was isolated for the first time by De Moura and coworkers from the methanolic extracts of the stem bark of *Z. rhoifolium*. The alkaloids dihydronitidine, 6-oxynitidine and skimmianine were also isolated along with. The bioassay of antiplasmodial activity of dihydroavicine, dihydronitidine, oxyavicine, oxynitidine, fagaridine, avicine and nitidine isolated from *Z. rhoifolium* bark, have shown that the last five possessed antimalarial activity and nitidine was the most potent, displaying an IC₅₀ < 0.27 μM against *Plasmodium falciparum*. A traditional remedy, the trunk bark decoction in water, also contained fagaridine, avicine and nitidine justifying the traditional use of *Z. rhoifolium* bark as antimalarial. Zanthoxyline, 8-acetonyldihydronitidine and 8-acetonyldihydroavicine were also found to have antibacterial activity. The alkaloids rhoifolines A and B were also isolated from the plant.
Zanthoxylum nitidum.

*Z. nitidum* (Roxb.) DC is a morphologically variable species, found as a liane in rain-forest and as a shrub in dryer habitats. It is widely distributed in India, China and Australia. Its fruit is used as a spice and the stems and leaves have been used as folk medicines to relieve toothache and sore throat. The root has been used to promote blood circulation, to dissipate blood stasis to treat traumatic injury and to cure snake bite. Pharmacognostic investigations of different parts of the plant have proved the antibacterial, antinociceptive, anti-inflammatory, antioxidant, antifungal, analgesic and anti-inflammatory activities. Apart from that, many of the compounds isolated from *Z. nitidum* also were found to exhibit antitumor, antiviral, antifungal, analgesic, and anti-inflammatory activities.

The presence of several types of alkaloids, especially benzophenanthridines, which are typical of the Rutaceae, have been reported in this plant. Some other types of secondary metabolites, including coumarins and lignans were also isolated from the root of *Z. nitidum*. From the Australian accession of *Z. nitidum* the alkaloids β-amyrin, dihydrochelerythrine, (-)-asirinin, (-)-sesamin, nonchelerythrine, decarine, arnottianamide, 6-methoxy-5,6-dihydrofagaridine, liriodenine, decarine acetate, hesperidin and nitidine were isolated. Yang and Chen reported five alkaloids, 5,6-dihydro-6-methoxynitidine, dictamnine, γ-fagarine, skimmianine and 5-methoxydictamine from the roots of *Z. nitidum*. Compounds 5,6-dihydro-6-methoxynitidine, skimmianine and 5-methoxydictamine showed invitro anti viral effect against hepatitis B virus and compounds dictamnine, γ-fagarine and 5-methoxydictamine showed marked antimitotic and antifungal activity. Skimmianine, a furoquinoline alkaloid with its strong acetylcholinesterase inhibiting activity is relevant to the treatment of Alzheimer’s disease. The ethanolic extract of the rhizome
of the *Z.nitidum* was analyzed phytochemically and two new benzophenanthridine alkaloids, 8-methoxyisodecarine and 8-methoxysanguinarine were obtained\(^2^7\). Terihanine was isolated by preparative paper chromatography technique from the root bark of *Z. nitidum* by Tsai and coworkers. They also obtained decarine, arnottianamide, toddaquinoline and 6-carboxymethylidihydrochelerythrine from the phenolic tertiary base fraction of the stem bark\(^2^8\).

In a study on the bark of the plant, Yang and coworkers isolated five novel alkaloids viz, zanthomuurolanine, epi-zanthomuurolanine, zanthocadinanines A and B and epi-zanthocadinanine B\(^2^3\). (R)-(+-)-Isotembetarine, a quaternary alkaloid was obtained by a combination of ion-pair extraction and preparative ion-pair HPLC using sodium perchlorate from the stems of *Z. nitidum*\(^2^9\).

The total alkaloid contents obtained from *Z.nitidum* were characterized by HPLC coupled with Tandem Mass Spectrometry and ten alkaloids were identified by Cai and coworkers. The fragmentation mechanism of six of them namely, dihydronitidine, 8-acetonyldihydronitidine, 8-acetonyldihydrochelerythrine, nitidine and 1,3-bis(8-dihydronitidinyl)acetone were detailed in their communication\(^3^0\). The chemical profiles of alkaloids of the plant were reported by Liang and coworkers as nitidine, berberrubine, coptisine, chelerythrine, oxyavicine, dihydrochelerythrine, iridiodenine and 6,7,8-trimethoxy-2,3-methylendioxybenzophenanthidine using HPLC/MS. The report also indicated that the contents of alkaloids vary significantly from habitat to habitat\(^3^1\).

Protuberine alkaloids and coptisine and berberrubine were reported for the first time in the family of Rutaceae in a study conducted by Hu and coworkers on *Z. nitidum*\(^3^2\). Hu and coworkers in a different study, obtained two new alkaloids from this plant species namely 4,5-dihydroxy-1-methyl-3–
oxo-2(trichloromethyl)-3H-indolium chloride and 4-(2-methoxyethyl)N,N-dimethylbenzenamine. They isolated these compounds from the ethanolic extract of the roots. Hu and coworkers also evaluated the bioactivity of benzophenanthridine alkaloids of the plant and showed that nitidine, dihydrochelerythrine, oxyavicine, 8-methoxychelerythrine and 8-hydroxydihydrochelerythrine exhibited equivalent analgesic and anti-inflammatory effects as hydrocortisone.

Nitidine and fagaronine isolated from the plant were also characterized as inhibitors of topoisomerase I. In common with the antitumor agent camptothecin, both nitidine and fagaronine were found to stabilize the covalent binary complex formed between calf thymus topoisomerase I and DNA. Both nitidine and fagaronine inhibited the topoisomerase I-mediated relaxation of supercoiled pSP64 plasmid DNA more effectively than camptothecin; unlike camptothecin, both of these benzophenanthridine alkaloids also bound directly to and mediated the unwinding of B-form DNA. In this report by Wang and coworkers, nitidine and fagaronine were also studied in comparison with camptothecin to determine the sequence specificity of DNA breaks produced from a 32P-end-labeled duplex in the presence of topoisomerase I and they could establish that all three compounds produced very similar cleavage patterns. This study becomes relevant due to the fact that DNA topoisomerases are enzymes that alter the topological state of DNA, which is required for certain critical cellular processes such as DNA replication and transcription.

A very recent report in the Food chemistry journal claims the isolation of new benzenoids and anti-inflammatory constituents from Z. nitidum along with many other compounds. Here Chen and coworkers described the isolation and characterization of \((E)-4-(4\text{-hydroxy-3-methylbut-2-enyloxy})\text{benzaldehyde, (E)-methyl 3-}(4-(E)-4\text{-hydroxy-3-methylbut-2-}

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enyloxy)phenyl)acrylate, and (Z)-methyl3-(4-((E)-4-hydroxy-3-methylbut-2-enyloxy)phenyl)acrylate from the stem wood of *Z. nitidum*, together with 17 known compounds which include, the benzo[c]phenanthridines, 6-acetonyldihydrochelerythrine 6-acetonyl-8-O-demethylidihydrochelerythrine and decarine, the coumarins, aesculetin dimethyl ether and scopoletin, four lignans, (+)-episesamin, (+)-sesamin, piperitol3,3-dimethylallylether, and sesaminone, N-methylflindersine and γ-fagarine\(^{36}\).

**Zanthoxylum leprieurii**

This is a small to large tree with pale grey stems and branches having straight or curved spines on them. They are found on alluvial soils in hot, dry river valleys near pans, on termite mounds and in the understorey of evergreen forest mainly in the African continent\(^{37}\).

Dried fruits of *Z. leprieurii* are used as spices by local population in many regions of Africa\(^{38}\) and the essential oils from the stem and root bark of this plant is found to show antifungal activity\(^{39}\).

Chemical investigation of the roots and fruits of *Z. leprieurii* led to the isolation of three new alkaloids including two acridone derivatives, helebelicine A and B and 10-O-demethyl-12-O-methylarnottianamide together with thirteen other compounds including many other acridone derivatives, hesperidine, nitidine, angoline, lupeol, dehydronitidine, decarine and sesamine. The brine-shrimp (*Artemia salina*) lethality bioassay of the chloroform extract of the fruits showed modest cytotoxicity with LD\(_{50}\) at 13.1µg/mL. Four acridone alkaloids isolated in this study were found to be moderately active against lung carcinoma cells (A549), colorectal adenocarcinoma cells (DLD-1) and normal cells (WS1) with IC\(_{50}\) values ranging from 27 to 77µM among which 1-hydroxy-3-methoxy-10-methyl-9-acridone was most active in contrast to the positive control etoposide used\(^{40}\).
Arborine and xanthoxoline, isolated from the *Z. leprieurii* are reported to display good invitro antiplasmodial activity against 3D7 strains of *Plasmodium falciparum* with IC\(_{50}\) values of 4.5±1.0 and 4.6± 0.6μg/mL. The same study reports the isolation of tegerrardin, which along with arborine, is a good chelating agent with 90% and 61% radical scavenging activity respectively\(^{41}\).

**Zanthoxylum riedelianum**

A new benzophenanthridine alkaloid, 6-acetonyl-N-methyl-dihydrodecarine was isolated from *Z. riedelianum* by Fernandes and coworkers together with lupeol, 6-acetonyldihydrochelerythrine and 6-acetonyldihydroavicine\(^ {42}\).

**Zanthoxylum scandens**

A report on the isolation of the chemical constituents of the stem bark of *Z. scandens* gave one new dioxoaporphine alkalo id, zanthodione together with 19 known compounds. The \(\gamma\)-fagarine and (+)-platydesmine, which are among these isolates, were found to be very potent anti- HIV constituents\(^ {43}\).

A new phenylpropanoid ester, (E)-O-geranylconiferyl alcohol (9Z, 12Z)-linolate was isolated from the bark of *Z. scandens* from Vietnam. (E)-O-geranylconiferyl alcohol and the alkaloids, norchelerythrine, magnoflorine and (−)-(S)-O-methylbalfourodinium cation were also obtained along with\(^ {44}\).

**Zanthoxylum flavum**

A Natural Product Communications report, which demonstrates the bio-guided fractionation of the methanolic extracts of the roots of *Z. flavum*, yielded 18 constituents, which includes seven coumarins, seven alkaloids, one lignan, two sterols and one triterpene. Among these 14 compounds were
identified for the first time from this plant. The coumarine oxypeucedanin displayed significant antioxidant activity in the cell-based DCFH-DA assay\textsuperscript{45}.

\textit{Zanthoxylum integrifoliolum}

\textit{Z. integrifoliolum} (Merr.) Merr. is a large evergreen tree and its bark is traditionally utilized as remedy for snake-bite and is found to be a good source for antiplatelet agents such as chelerythrine and avicine pseudocyanide. The alkaloids of benzo[c]phenanthridines, quinolines, and triterpenoids are the major constituents of this plant from the past reports\textsuperscript{50}.

Chen and coworkers in 2007 reported five new compounds which include two new phenylpropenoids, two new bis(1-phenyl ethyl)phenols and a new bis-quinolinone alkaloid from the stem wood of \textit{Z. integrifoliolum} together with 17 known compounds. Among these isolates, N-methylflindersine, (-)-simulanol and evofolin-C exhibited potent inhibition against induced super oxide production. Antiplatelet aggregation, vasorelaxing, anti-inflammatory activity and cytotoxic activities have also been demonstrated for some of these compounds\textsuperscript{46}.

Chen and coworkers in a different report, presented three new alkaloids 7,8-dehydro-1-methoxyrutaecarpine, isodecarine and 8-dimethoxy chelerythrine together with sixteen other known compounds from the plant. They have also reported the cytotoxic evaluation of these compounds\textsuperscript{47}.

Examination of the tertiary non-phenolic base fraction of the leaves of \textit{Z. integrifoliolum} led to the isolation of a new bishordeninyl alkaloid, integramine along with alfileramine, γ-allocryptopine, pseudoprotopine and (+)-sesamin\textsuperscript{48}.

Investigating the chemical constituents and anti-platelet aggregation principles obtained from the fruit of the plant resulted in the isolation of three
new isobutylamides, lanyuamide I-III and six known isobutylamides, tetrahydrobungeanool, γ-sanshool, hydroxy γ-sanshool, mixture of $(2E,4E,8Z,11Z)-$ and $(2E,4E,8Z,11Z)-2'$-hydroxy-N-isobutyl-2,4,8,11-tetradecatetraenamide and hazaleamide which was mixed with lanyuamide III$^{49}$. Four new compounds including two new lignans, (+)-pinoresinol-di-3,3-dimethylallyl ether and (+)-pinoresinol-3,3-dimethylallyl ether; zanthonitrile and one new flavonoid, 3,5-diacetyltambulin and 18 known compounds which include (-) tetrahydroberberine, skimmianine, canthin-6-one, rutaecarpine, norchelerythrine, nornitidine, decarine, atanine, tambulin, prudomestin, scopoletin and (+)-piperitol-3,3-dimethylallyl ether were also isolated from the CHCl$_3$-soluble fraction of the fruit. Among these isolates, 13 compounds showed strong in vitro antiplatelet aggregation activity, with only (-)-tetrahydroberberine showing weak vasorelaxing effect in high potassium- or norepinephrine- induced contraction of rat aorta$^{50}$.

Two new compounds, tridecanonchelerythrine, a benzo[c] phenanthridine alkaloid with an interesting long chain of the 2'-oxotridecyl group at position 6 and conifegerol which is fragrant and an ether of a coniferyl alcohol and geraniol, were isolated from the bark of Z. integrifoliolum$^{51}$.

**Zanthoxylum madagascariense**

Fractionation of the cyclohexane extract of the stem bark powder of Z. madagascariense led to the isolation of a new benzophenanthridine type alkaloid, rutaceline that was evaluated for its anti proliferative capacity on the human colorectal adenocarcinoma and the African green monkey kidney (Vero) cell lines. Rutaceline also induced DNA fragmentation and a dose dependant clastogenic effect in both cell lines$^{52}$. 

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**Zanthoxylum chiloperone**

Z. chiloperone var. angustifolium Engl. (syn Z. rugosum St.-Hil & Tul.) is used in traditional medicine to treat fungal and protozoal infections in the central area of South America.

The alkaloids candicine, tembetarine, magnoflorine, laurifoline, chelerythrine, and nitidine were isolated from the cortex of *Z. chiloperone*, collected in Argentina. The concentrated dichloromethane extract of the dried leaves of the plant gave 5-methoxycanthin-6-one and anisocoumarin. From the roots, Canthin-6-one and 5-methoxy-canthin-6-one, trans-avicennol, trans-avicennin, chelerythrine and skimmianine were isolated\(^5\). On exploring the anti-plasmodial effects of compounds isolated from *Z. chiloperone*, the pyranocoumarin transavicennol and the canthinone alkaloids, canthin-6-one and 5-methoxycanthin-6-one were found to have IC\(_{50}\) of 0.5-2.7, 2.0-5.3 and 5.1-10.4\(\mu\)g/mL, respectively on chloroquine/mefloquine resistant and sensitive strains of *P. falciparum*. Moreover, the formation of heme adducts by these compounds is described by a novel alternative method based on MS-CID methods. The alkylamide sanshool was also identified, for first time in this plant\(^4\). Canthin-6-one possesses a broad spectrum of antifungal and leishmanicidal activities. This compound exhibited trypanocidal activity in vivo in the mouse model of acute or chronic infection. Considering the low toxicity of this compound, it could prove advantageous compared to the current chemotherapy of Chagas disease\(^5\). Canthin-6-one and 5-methoxy-canthin-6-one also exhibited antifungal activity against *Candida albicans*, *Aspergillus fumigates* and *Trichophyto mentagrophytes*\(^6\).

**Zanthoxylum schinifolium**

*Z. schinifolium* is an aromatic plant that is distributed in north-eastern Asia such as Korea, China and Japan. The leaves, fruits, seeds and roots of *Z.
The chemical constituents of the leaves of this species include 50 known compounds and a new amide, cis-fagaramide. The known compounds comprise 21 coumarins, 11 alkaloids, 1 furan, 3 chlorophylls, 1 diterpenoid, 1 sesquiterpenoid, and 4 steroids. A cytotoxic coumarin, 7-[(E)-3',7'-dimethyl-6'-oxo-2',7'-octadienyl]oxy-coumarin together with three known compounds, schinilenol, schinindiol and 7-[(E)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy]-coumarin were also isolated from the leaves.

The study of the chloroform soluble portion of the root bark led to the isolation of 8 new coumarins along with 26 known compounds. The known ones include 14 coumarins and 9 alkaloids. Among the isolates terpenyl coumarins and furoquinolines were active constituents with antiplatelet aggregation in vitro and collinin showed significant anti-HBV DNA replication activity.

In an analysis of several herbs to find the immune modulator with biological safety, the results for *Z. schinifolium*-repeated administration enhances B cell immunity and is biologically safe conclude that *Z. schinifolium* is one of the safe candidates to modulate immunity.

Screening for insecticidal principles from several Chinese medicinal herbs showed that the fruit pericarp of *Zanthoxylum schinifolium* possessed significant feeding deterrence against two stored product insects, *Tribolium castaneum* and *Sitophilus zeamais*. From the methanol extract, two feeding
deterrents were isolated by bioassay-guided fractionation and the compounds were identified as schinifoline and skimmianine\textsuperscript{61}.

The composition of the essential oils of the fruit of \textit{Z. schinifolium} was analyzed by GC-MS and linalool (29\%) was found to be the major aroma component, followed by limonene (14\%) and sabinene (13\%), the tingling sensation of the preparations from this fruit is caused mainly by the alkylamide hydroxy-\textalpha-sanshool\textsuperscript{62}.

\textit{Zanthoxylum usambarenses}

The bark and leaves of \textit{Z. usambarenses} have been used traditionally in the treatment of severe colds and to alleviate stomachache and toothache. Bioassay guided fractionation of the dichloromethane extract of the roots and bark of \textit{Z. usambarenses} led to the isolation of two physiologically active compounds, canthin-6-one, which act as fungicide and pellitorine, which has insecticidal property. Oxychelerythrine, norchelerythrine, (+)-sesamin and (+)-piperitol-3,3-dimethylallyl ether were also isolated and for the first time from this plant\textsuperscript{63}.

From the stems of \textit{Z. usambarenses}, (+)-tembetarine, (+)-magnoflorine, (+)-\textN-methylplatydesmine, (-)-oblongine, (-)-\textit{cis-N}-methylcanadine, nitidine, chelerythrine, (-)-usambarine, and (-)-edulinine and a new alkaloid usambanolone perchlorate were isolated\textsuperscript{64}.

\textit{Zanthoxylum dimorphophyllum}

The new diprenyl coumarin dimoxylin along with scoparone and the alkaloids oxyavicine, canthin-6-one, 4,5-dihydro-canthin-6-one, chelerythrine, norchelerythrine and \textgamma-fagarine were isolated from the \textit{Z. dimorphophyllum} bark\textsuperscript{65}. 

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Zanthoxylum tetraspermum

*Z. tetraspermum* is utilized in the indigenous system of medicine in Sri Lanka for the treatment of dyspepsia, rheumatism and some forms of diarrhea. The alkaloids 8-acetonyldihydronitidine and 8-acetonyldihydroavicine were isolated from the stem bark of *Z. tetraspermum* along with β-sitosterol, β-amyrin, iso-arborenone, betulinic acid, α-amyrin, spathulenol, liriodenine, sesamin, lichexanthone and (+)-piperitol-γ-γ-dimethylallyl ether. Both 8-acetonyldihydronitidine and 8-acetonyldihydroavicine showed strong antibacterial and antifungal activities.

Zanthoxylum caudatum

*Z. caudatum* is a rare plant endemic to the Sri Lankan islands. There are no reports of ethno medical uses for the plant presumably due to its extreme rarity. *Z. caudatum* bark extract was found to contain β-sitosterol, stigmasterol, sesamin, savinin, liriodenine, decarin and 8-O-desmethyl-N-nornitidine. Savinin exhibit potent spermicidal activity. Both sesamin and savinin also show insecticidal activity. Savinin also have potent cytotoxic effects. The presence of biologically active principles corroborates the significance of the plant in the area of medicine.

Zanthoxylum avicennae

*Z. avicennae* (Lam.) DC is an evergreen shrub and a decoction of its stems is used as a stomach tonic and as a counter-poison to snake bite. The research on this plant has resulted in the isolation of flavonoids, alkaloids, coumarins and terpenoids.

The bishordeninyl terpene alkaloids, (-)-culantraramine and (-)-culantraminol, (-)-culantraramine N-oxide, (-)-culantraminol N-oxide and
avicennamine have been isolated from methanol extract of the leaves of *Z. avicennae*.

The methanol extract of *Z. avicennae* inhibited FMLP/CB induced superoxide anion generation and elastase release in a concentration-dependent manner with IC$_{50}$ values of 6.34 ± 0.56 and 15.32±1.46μg/mL respectively. The search for compounds with anti-inflammatory activities led to the isolation of eight new compounds, including four new neolignans, (7′$S$,8′$S$)-bilagrewin, (7′$S$,8′$S$)-5-demethoxybilagrewin, (7′$S$,8′$S$)-5-O-demethyl-4′-O-methylbilagrewin, and (7′$S$,8′$S$)-nocomtal, a new coumarinolignan, (7′$S$,8′$S$)-4′-O-methylcleomiscosin, two new lignan derivatives, (+)-9′-O-(Z)-feruloyl-5,5′-dimethoxylariciresinol and (+)-9′-O-(E)-feruloyl-5,5′-dimethoxylariciresinol, and a new chromene, (E)-3-(2,2-dimethyl-2H-chromen-6-yl)prop-2-enal from the stem wood of *Z. avicennae*, together with 18 known compounds. Among the isolated compounds, (7′$S$,8′$S$)-4′-O-Methylcleomiscosin, cleomiscosin, skimmianine, robustine and integrifoliolin exhibited inhibition (IC$_{50}$ ≤ 18.19 μM) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasinB (FMLP/CB). In addition, skimmianine inhibited FMLP/CB-induced elastase release with an IC$_{50}$ value of 19.15 ± 0.66 μM.

**Zanthoxylum culantrillo**

*Z. culantrillo* (H.B.K.) Krug and Urban is a medium to large tree distributed throughout Central and South America. Leaves and bark of this plant are used by the native population of the Baudo region for the preparation of a textile dye. The Phytochemical investigation by Swinehart and Stermitz has shown the presence of eight alkaloids and two lignans and from the ethanolic extract of leaves of *Z. culantrillo*, methylpiperitol, sitosterol, skimmianine, afzelin and quercitrin were isolated while the stem
bark yielded methylpiperitol, (+)-sesamin and the new THF lignan, 3,4-dimethoxy-3’,4’-methylenedioxy-7,9’-epoxylignan-9-ol\textsuperscript{71}.

**Zanthoxylum piperitum**

*Z. piperitum* DC (Japanese pepper) is a deciduous and shrubbery tree with characteristic citrus like flavor. The whole plant and its pericarp are commonly used as a spice. *Z. piperitum* leaf extract is used as a diuretic in traditional Asian medicine for treating vomiting, diarrhoea and abdominal pain. *Z. piperitum* has been used as an antibacterial agent, as a cholesterol acyltransferase inhibitor and for hepatoprotection. Aliphatic acid amides isolated from *Z. piperitum* relax gastric body circular muscles and contract ileum and distal colon longitudinal muscles and methanol extracts of this plant inhibit bromobenzene-induced lipid peroxidation in the rat liver\textsuperscript{72}. It has been reported that the fruit extract of the the plant has strong antioxidant activity\textsuperscript{73}. The fruit extract is also used as insecticide and insect feeding deterrents\textsuperscript{74}.

*Z. piperitum* essential oil contains myrcene, octanal, \textit{d}-limonene, linalool, citronellal, geraniol, phellandral, sabinene and geranyl acetate\textsuperscript{75}. The oil has been shown to repel mosquitoes and inhibit growth of foodborne pathogens such as *Vibrio parahaemolyticus*\textsuperscript{72}. This plant’s beneficial effects also have been traditionally associated with antifungal, antilipid, peroxidative and antibacterial activities. Recently, it was reported that *Z. piperitum* has antiviral and anti-inflammatory activities\textsuperscript{80}.

Six aliphatic acid amides were isolated from the pericarp of *Z. piperitum* fruits by Hatano and coworkers and since the unsaturated fatty acid amide constituents are known to be associated with pharmacological properties, their presence may be related to the medicinal properties of *Z. piperitum*\textsuperscript{76}. Five linear fatty acid amides of the sanshool class were also
isolated from seeds which exhibited weak cytotoxicity in the A-549 (human 
lung cancer) cell line. One of the alkylamides, HO-α-sanshool is found to 
stimulate trigeminal sensory neurons in the rat by inducing firing in tactile and 
cool sensitive neurons as well as sensitivity to innocuous stimuli in previously 
insensitive neurons. The neurons that respond to HO-α-sanshool do not 
belong to the major class of chemically sensitive pain receptive neurons, the 
polymodal nociceptors. Hence it is suggested that the oral sensations caused 
by Z. piperitum depend on neurons, which may mediate sensitivity to most 
irritants, indicating the possibility of this plant derived compounds being 
useful as feeding deterrents for mammals.

An assessment of mutagenicity and toxicity is necessary to ensure the 
relatively safe use of any plant-derived medicine. The evaluation of the 
potential health risks of Z. piperitum derived essential oil by testing the 
mutagenicity and toxicity based on bone marrow micronucleus, bacterial 
reverse mutation, and chromosomal aberration tests provided results to 
indicate that ingesting the essential oil produces no bone marrow 
micronucleus abnormalities, mutagenicity, or chromosomal aberration thereby 
assuring its safe use in dietary supplements or functional ingredients.

Z. piperitum leaf due to the presence of the phenolics hyperoside, 
quercitrin, afzelin, and quercetin, exhibit strong antioxidant activities and 
neuronal cell protective effects. So it can be utilised as effective and safe 
functional food substance as a natural antioxidant that also reduce the risk of 
neurodegenerative disorders.

Zanthoxyllum davyi

Z. davyi (I. Verd.) Waterm. (syn. Fagara davyi I. Verd.) is a medium to 
tall tree which is traditionally employed in the treatment of snakebite and 
severe coughs and colds. Its spines are used for infected wounds, the leaves
for chest pains and the stem bark to treat boils, pleurisy and toothache. Root preparations are used for mouth ulcers, sore throats and as an aphrodisiac and root bark decoctions are used as a tonic both for man and animals and to treat toothache.

The stem bark of this South African ethnomedical tree yielded five benzo[c]phenanthridine alkaloids, chelerythrine, dihydrochelerythrine, bocconoline, 6-hydroxydihydrochelerythrine and 6-methoxy-7-dimethylhydrochelerythrine together with 4-methoxy-1-methyl-2(1H)-quinolinone and the uncommon lignan meso-sasamine. Chelerythrin is a well-documented antimicrobial and anti-inflammatory agent.

The alkaloid pellitorine is also reported from the stem bark, isolated along with methyl octadecyl ketone, lupeol and hesperidin. The alkaloid skimmianine was isolated from the leaves.

**Zanthoxylum quinduense**

Phytochemical investigations on *Z. quinduense* Tul. [syn. *Fagara quinduense* (Tul.) Engl. *F. macrosperma* (Tul.) Engl., *Z. macrospermum*(Tul.)] led to the isolation of six benzophenanthridine alkaloids, 8-hydroxyl-2,3-methylenedioxy-9-methoxybenzophenanthridine, norchelerythrine, 6-acetonyldihydrochelerythrine, normitidine, arnottianamide and decarine, one lignin syringaresinol, one phenylpropene, evofolin-C, two benzenoids p-hydroxybenzaldehyde and vanillic acid, three sterols β-sitosterol, stigmasterol and campesterol and lupeol and a mixture of saturated and unsaturated fatty acids, and their derived methyl esters from extracts of the wood and bark. The new alkaloid 8-hydroxy-2, 3-methylenedioxy-9-methoxybenzophenanthridine and the compounds (-)-6-carboxymethyl dihydrochelerythrine, chelerythrine, berberine, N-methyltetrahydro
columbamine and N-methyltetrahydropalmatine, (-)-xylopinididine and isotembetarine\textsuperscript{84} were also obtained from the bark of \textit{Z. quinduense}.

\textbf{Zanthoxylum buesgenii}

The alkaloid buesgeniine was for the first time isolated from \textit{Z. buesgenii}. In addition to this compound, decarin and three lignans namely sesamine, matairesinol dimethylether and methylpluviatilol, were also identified\textsuperscript{85}.

\textbf{Zanthoxylum heitzii}

This species is a shrub of humid rain forests and it is traditionally used as medicinal plant against cancer, syphilis, malaria, cardiac palpitations as well as urogenital infections\textsuperscript{86}. The isolation of constituents from the roots of this traditional medicinal plant of central Africa, has been done by extracting in dichloromethane and methanol and analyzing them. This work is reported to be the first in isolation of 6-methylnitidine chloride along with skimmianine, lupeol, lupeone and β-sitosterol. Flindersine was isolated from heartwood of \textit{Z. heitzii} and other metabolites including terpenes, lignans and alkaloids (fagaramide, nitidine) were isolated from the bark\textsuperscript{87}.

Mbaze and coworkers isolated from the stem bark of \textit{Z. heitzii}, two amides, heitziamide A and heitziamide B and two phenylethanoids, heitziethanoid A and heitziethanoid B together with thirteen known compounds, trans-fagaramide, arnottianamide, iso-γ-fagarine, isoskimmianine, arctigenin methyl ether, savinin, (+)-eudesmin, (+)-sesamin, lupeol, lupeone, β-sitosterol, stigmasterol and stigmasterol-3-O-β-D-glucopyranoside. Among them, nine compounds were evaluated for oxidative burst inhibitory activity in a chemoluminescence assay and for cytotoxicity against PC-3 prostate cancer cells. All compounds exhibited a clear suppressive effect on phagocytosis response upon activation with serum
opsonized zymosan at the range of IC\textsubscript{50} of 2.0-6.5\textmu M, but no cytotoxic effect was observed (IC\textsubscript{50} > 100\textmu M)\textsuperscript{88}.

**Zanthoxylum syncarpum**

A new (+)-norepinephrine derivative, syncarpamide along with (+)-S-marmesin and decarin have been isolated from the stem of *Z. syncarpum*. Syncarpamide and decarin showed antiplasmodial activity against *Plasmodium falciparum*\textsuperscript{89}.

**Zanthoxylum clava-herculis**

Gibbons and coworkers demonstrated antibiotic activity against methicillin- resistant *Staphylococcus aureus* associated with extracts of the bark of the plant. The bioassay-guided isolation of the alkaloid extract led to the characterization of chelerythrine as the major active principle\textsuperscript{90}.

The lignoid compound asarinin and the insecticidal and sialogogic compound neoherculin were also isolated from the bark of *Z. clava-herculis*. In a study of bioactive plants, it was found that the extract of the bark of the plant to be highly toxic to fish, and a fractionation undertaken based on this activity led to the isolation of (-)-asarinin, (-)-seamin, (-)-pluviatilol-\gamma,\gamma, dimethylallyl ether, neoherculin and N-acetylanonaine. Of these, the last two were found to be responsible for the toxicity of the extract and neoherculin was the major ichthyotoxic principle among them with an IC\textsubscript{50} of 2 X 10\textsuperscript{-7}M\textsuperscript{91}.

**Zanthoxylum simulans**

Many constituents that showed anti-platelet aggregation activity in vitro were isolated from the stem wood of *Z. simulans*. The pyrrole alkaloid, pyrrolezanthine[5-hydroxymethyl-1-[2-(4-hydroxyphenyl)ethyl]-1H-pyrrole-2-carbaldehyde], the lignan, (-)-simulanol[4-[3-hydroxymethyl-5-((E)-3-hydroxypropenyl)-7-methoxy-2,3-dihydrobenzofuran-2-yl]-2,6-dimethoxy-
phenol], γ-pyrone, zanthopyranone and many other known compounds were also obtained from the stem bark.

Chen and coworkers isolated two benzdelphenanthridine alkaloids, 6-methyldihydrochelerythrine and 6-methylnorchelerythrine together with 23 known compounds from the root bark of *Z. simulans*. Among them, the pyranquinoline alkaloids, zanthosimuline and huajiaosimuline exhibited cytotoxic activity. In addition, huajiaosimuline showed significant antiplatelet aggregation activity and induced terminal differentiation with cultured HL-60 cells. Chen and coworkers have also isolated three pyranoquinoline alkaloids from the stem bark of *Z. simulans* namely simulenoline, peroxysimulenoline, and benzosimulan together with 22 known compounds. In this study they also isolated and elucidated the structure of the known compound zanthodioline.

**Zanthoxylum hyemale**

The crude extract of the bark of *Z. hyemale* was associated with antispasmodic activity and the phytochemical analysis of this extract yielded three new compounds namely two quinoline alkaloids, (-)-R-Geilbalansine and hyemaline and the aromatic amide, N-[(2-(3,4-dimethoxyphenyl)-2-methoxyethyl)-2-methoxyethyl]benzamide(O-methylbalsamide) along with seven known compounds. Quinoline alkaloids, (-)-R-geilbalansine and hyemaline were also isolated from the stem barks of *Z. hyemale*.

The essential oils from aerial parts of juvenile leaves, mature leaves, fruit and flowers of *Z. hyemale* were isolated by hydrodistillation and analyzed by GC, GC-MS and chiral phase gas chromatography (CPGC). The major constituent of the juvenile leaf essential oil was the sesquiterpene trans-nerolidol (51%) while the main constituent of mature leaf and flower oils was
31 and 22% hyemalol, respectively. In the fruit, the most abundant components were the monoterpenes β-pinene (25%) and α-pinene (10%)\textsuperscript{96}.

**Zanthoxylum arborescens**

The first report of 2,5-dibenzyl-1,4-dimethylpiperazine in nature came from Grina and coworkers from the leaves of *Z. arborescens* which is also the first recorded occurrence of the potent hallucinogen N,N-dimethyltryptamine in the genus *Zanthoxylum*. They also obtained in their study, the new furanoquinoline dimethylpiperazine-8-hydroxy-4,7-dimethoxyfuranoquinoline along with 6-D-glucopyranoside of hordenine which was previously known only as a synthetic and the known alkaloids skimmianine, tembetarine, hordenine and N-methyltryptamine, from various parts of the plant\textsuperscript{97}.

**Zanthoxylum bungeanum**

The fruit of *Z. bungeanum* Maxim. is a peppery spice used in cooking and the pericarp of seeds in addition to being a widely used pungent condiment and seasoning, has also been employed as a traditional drug in the Indian and Chinese system of medicine for the treatment of vomiting, toothache, stomachache, abdominal pain owing to roundworm ascariosis, diarrhoea and dysentery\textsuperscript{102}.

The seed oil also demonstrated marked antioxidant activity in the DPPH radical-scavenging assay\textsuperscript{98}.

Unsaturated alkylamides, tetrahydrobungeanol, dihydrobungeanol, dehydro-7-sansho, bungeanol and isobungeanol and 7-sansho, flavonol glucosides, *viz.* quercetin 3’,4’-dimethyl ether-7-glucoside and tamarixetin-3,7-bisglucoside and the compounds hyperin, quercetin, quercitrin, foeniculin, isorhamnetin-7-glucoside, rutin, 3,5,6-trihydroxy-7,4’-dimethoxyflavone,
arbutin, sitosterol fl-glucoside, L-sesamin and palmitic acid were isolated from the pericarps of the fruit of the plant\textsuperscript{99,100}. Linalyl acetate, linalool, limonene, eucalyptol, β-myrcone, α-pinene, and piperitone are the major components of the essential oil extracted from the pericarp of \textit{Z. bungeanum}\textsuperscript{101}. The dichloromethane extract of the fruits of \textit{Z. bungeanum} was found to be highly repellent to insects. The active components were identified as three active monoterpenes, piperitone, 4-terpineol, and linalool. Piperitone is more repellent than is the common insect repellent N,N-diethyl-m-toluamide (DEET)\textsuperscript{102}.

\textit{Zanthoxylum chiriquinum}

\textit{Z. chiriquinum} Standl. is a very rare species and the dry leaves collected from Costa Rica were found to contain the bishordeninyl terpene alkaloid, O-methylalfileramine along with alfileramine, culantraramine and isoalfileramine\textsuperscript{103}.

\textit{Zanthoxylum coco}

\textit{Z. coco} Gill. [\textit{Fagara coco} (Gill.) Engl.] is a small tree whose chemistry is well worked upon. The alkaloids of the bark and foliage of \textit{Z. coco} have been studied for more than fifty years, and the quaternary phenethylamine candicine, the quaternary aporphines magnoflorine and N-methylisocorydine, the protoberberines, berberine and palmatine, the benzophenanthridines, chelerythrine and nitidine, the protopine bases, allocryptopine and fagarine and the furoquinolines skimmianine and γ-fagarine are all known constituents of this plant. The coumarin aurapten and the angular pyranoquinoline flindersine were also found to be present in the species\textsuperscript{104}. 
**Zanthoxylum Gillettii**

The chemical investigations of *Z. gillettii* Waterm. [syn. *Fagara macrophylla* (Planch ex. Oliv.) Engl.,] has yielded the skimmianine, dictamine, fagaramide, chelerythrine, dihydrochelerythrine, nitidine, arnotianamide, tembetarine, N-methyl-corydine, N-isobutyl-(2E,4E)-dodecadienamide, lupeol, γ-sanshool, and sitosterol as its constituents\(^{105}\). Various aliphatic and aromatic amides, the coumarins, scoparone and xanthotoxin, the lignans, sesamin, asarinin, savinin, hinokinin, arctigenin and matairesinol are also reported from the root and stem bark of the plant\(^1\).

**Zanthoxylum naranjillo**

From the hexane crude extract of the leaves of *Z. naranjillo*, Bastosa and coworkers isolated fourteen compounds which include the three sesquiterpenes, β-selinene, α-cyperone and (-)-juniper camphor, four furofuran lignans, (+)-sesamin, (-)-pluviatylol-4′-O-γ,γ-dimethylallyl ether, (-)-xanthoxylol-4′-O-γ,γ-dimethylallyl ether, (+)-piperitol-4′-O-γ,γ-dimethylallyl ether, four dibenzylbutyrolactone lignans, (-)-hinokinin, (-)-hibalactone, (-)-methylpluviatolide, (-)-kaerophylin, two furan lignans, (-)-cubebin and (-)-3,4-dimethoxy-3,4-desmethylenedioxy-cubebin and sitosterol\(^{106}\).
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2. A REVIEW ON ZANTHOXYLUM RHESTA

Z. rhesta DC, Syn Z. budruga wall Syn. Z. limonella (Dennst) Alston, commonly known as mullilam, is a small or moderate sized deciduous evergreen tree with pale corky bark, covered with conical prickles on stems and branches and belong to family Rutaceae. The leaves are imparipinnate; leaflets 7 to 9, opposite, ovate- lanceolate, shining and glandular-crenate. Flowers are white to yellowish-white in terminal paniculate cymes. Fruit are of 4 globose cocci with seeds that are bluish-black and shining. The plant is usually found in evergreen forests of Assam, Meghalaya and the Eastern and Western Ghats of Peninsular India. It is also widely distributed throughout Bangladesh, Malaysia and other parts of South Asia\(^1\)\(^-\)\(^3\).

Z. rhesta, as the other species of the family Rutaceae, has also found its niche in traditional system of medicines. The fruits and stem bark of this plant are used traditionally as a stimulant, astringent, stomachic and digestive and prescribed for urinary infection, dyspepsia, heart troubles, tooth ache, asthma and bronchitis\(^4\). The Naga tribe in the North-Eastern region of India employs the leaf decoction in the treatment of intestinal-worm infections. A study conducted by Yadav and Tangpu on the therapeutic efficacy of Z. rhesta leaf extract on cestoda infections in rats revealed that the extract had a pronounced efficacy against the larval stage of parasite when compared to the immature or the adult stage\(^5\). The plant is also traditionally used as antidiabetes, antispasmodic, diuretic and anti-inflammatory agent in other regions of India\(^6\). Rahman and coworkers\(^7\) reported that the plant also bears significant antinociceptive and antidiarrheal activities.

The dried fruits of Z rhesta are used as condiments in both sweet and savoury preparations and have spice value and are digestive and appetizing\(^8\). The fruits of Z rhesta consist of aromatic carpels and inner seeds which
resemble black pepper in pungency. The essential oil obtained from the dried fruits is called mullilam oil\(^9\) which is concentrated in the pericarp and the seeds are rich in fatty oil\(^{10}\). The seed oil is effective in cholera and is useful as an antiseptic, disinfectant and anti-inflammatory agent\(^{11}\). The essential oil from the fruits is also reported to have anesthetic and hypotensive activities\(^{12}\).

The \(Z.\ rhetsa\) oils of leaves, fruits and seeds comprise of pinenes, sabinene, \(\alpha\)-terpinene, \(\beta\)-phellandrene, terpinen-4-ol, mullilamdiol, 1,4-cineol, cuminic aldehyde, octanal, decanal, cryptone and phlorophenone dimethyl ether\(^{13}\). Shafi and coworkers identified 118 compounds from the leaf essential oil of \(Z.\ rhetsa\), caryophyllene oxide(12.7%), \(\beta\)-caryophyllene (9.6%), \(\beta\)-copaene(5.3%) and sphathulene(3.3%) being the chief compounds\(^{14}\). An analysis of the aroma compounds of the essential oil of seeds from the south Indian \(Z.\ rhetsa\) was carried out by Jirovetz and coworkers. In their work they described the aroma of the essential oil of seeds as spicy (direction of pepper), herbal–earthy, weak–fruity (lemon-like: sweet-orange note), weak floral, weak green, pinene– and terpinene like with woody-smokey notes in the background. They also identified more than 40 compounds from the seed oil, the major ones being sabinene (47.12%), alpha-terpineol (7.73%), terpinen-4-ol (6.61%), beta-pinene (5.99%), limonene 4.06%), alpha-pinene 3.87%), gamma-terpinene (3.64%), alpha-terpinene (3.45%) and para-cymene (3.08%)\(^{13}\). Unlike oils from the seeds of Indian plant, the Australian variety was sesquiterpenoid in nature with \(\beta\)-caryophyllene (27.5%) and germacene D (18.4%) as the major constituents. The seed oil also was found to have 47.7% linolenic- oleic acids\(^{15}\) and the petroleum ether extract of the seed in an assay by Agarwal and coworkers gave an acid value of 36.05, iodine value 27.1, saponification value 192.03, acetyl value 47.9, solid acids 56.9% and unsaponifiable 0.85%. It also contained behenic acid (1.03%), arachidic acid (10.15%), stearic acid (32.56%), palmitic acid (11.06%), myristic acid (1.19%), oleic acid (86.36%) and linoleic acid (7.65%)\(^{10}\).
Previous chemical examinations of the non volatile constituents from different parts of the plant lead to the isolation of many quinoline and indopyridoquinazoline alkaloids, phytosterols and flavanones and terpenoids\textsuperscript{16}. Compounds isolated from \textit{Z. rhetsa} include dihydroavicine, rhetsinine\textsuperscript{17}, N-methylflindersine, zanthobungeanine, dictamine, rutaecarpine, γ-fagarine, skimmianine, evodiamine, canthin-6-one\textsuperscript{11}, rhetsine, rhetine and chelerythrine\textsuperscript{18} from bark; arborine and dictamine from fruits\textsuperscript{19} and rutaecarpine from seeds\textsuperscript{20}. The structures of the compounds are given in fig 2.2a.
Fig 2.2a Structures of compounds earlier isolated from *Z. rhetsa*
REFERENCES

3. PHYTOCHEMICAL ANALYSIS

Present work

The study presented in this section comprises of the phytochemical investigation of conical prickles on the stem bark of *Z. rhetsa*. This include the extraction of the dried plant material using different solvent systems, the fractionation and isolation of the chemical constituents, their purification and characterization of the individual compounds using various spectral techniques. The biological activity of the compounds and also the difference in activity due to chemical transformation of the functional groups present in them is also investigated.

2.3.1 Scope of the study.

From the literatures on the genus *Zanthoxylum* it is very much evident that all the species belonging to this genus are rich sources of a variety of chemical compounds with exceptional attributes. Not many are thoroughly studied and there is much more to be explored regarding their chemistry and biological activity. Kerala, God’s own country, is bestowed with a wide range of pharmacologically benign flora awaiting detailed exploration. Only few publications have come out on the chemistry of *Z. rhetsa* throughout the broad spectrum of scientific research, especially from South India, which makes the plant motivating and this study reasonable. The already reported compounds from *Z. rhetsa* mainly belong to the flavonoids, alkaloids and terpenes with interesting structural backbone and are found to possess remarkable properties. Not much has been done to understand how these compounds can be structurally altered to improve their potential. Also as the plants show marked regional variations with respect to the compounds present
in them, a novelty in the chemical profile or a change in the amount of a useful molecule can always be expected.

The conical prickles on the stem bark of the tree were selected for investigation. Such modifications on the exterior of the plants are expected to function in protection of the plants against adverse situations including pathogen attack. Hence the chemical compounds present in them are likely to be biologically active even at lower concentrations. It is also observed by the natives that only a few plants can grow beneath Z. rhetsa, pointing to the plausible allelopathic influence of the compounds of the plant. The hazardous side effects of the synthetic compounds in all sectors including medicine and agriculture utilities have rekindled the search for safer natural means and hence the exploration into the natural world to unearth the biologically benevolent material is worth undertaking.

2.3.2 Materials and Methods

Plant material

The conical prickles on the stem bark of Z. rhetsa were collected from the botanical garden, University of Calicut in the month of January 2007. It was authenticated by Dr. A.K. Pradeep, department of botany, University of Calicut. A Voucher specimen of the plant material has been deposited in the specially maintained herbarium in the Department of Chemistry, University of Calicut.

Melting Point Determination:

The melting points of all the crystalline isolates were determined using Toshniwal capillary melting point apparatus and are uncorrected.
Column Chromatography (CC)

Column chromatographic separation of the crude and semi purified extracts were carried out using Silica Gel (MERCK, 100-200 mesh) as the stationary phase. The columns were prepared as slurry with suitable solvents and a gradient elution was carried out by homogenous mixing of solvents with different polarity.

Thin Layer Chromatography (TLC)

Thin layer chromatographic plates were prepared using TLC grade Silica gel–G (MERCK) using Stahl apparatus.

Spray Reagents in TLC

1. 20 % aqueous Sulphuric Acid (20% $\text{H}_2\text{SO}_4$)

20 % aqueous Sulphuric acid was prepared, the sprayed plate was heated to 110°C until spots were visible.

With this reagent, the terpenoids developed brown, pink, purple or yellow colour.

2. Anisaldehyde –sulphuric acid reagent(ANS)

1ml Conc. sulphuric acid reagent was added to a solution of 0.5ml anisaldehyde in 50ml ethanol. This reagent is freshly prepared before use. The sprayed plate was heated to 110°C until spots were visible. It is a universal spray reagent and spots of different colours were observed for most of the components of the sample.

3. Dragendorff’s Reagent

Solution A: 0.85 g of basic bismuth nitrate is dissolved in a mixture of 10ml acetic acid and 40ml water.
Solution B: 8 g of potassium iodide in 20ml water

Stock solution: Equal volumes of A and B are mixed

Spray reagent: 1ml stock solution is mixed with 2ml acetic acid and 10ml water before use.

On spraying the plates with this reagent orange spots developed when nitrogen compounds or alkaloids were present. Spots intensified when sprayed later with HCl, or 50% water-phosphoric acid.

4. 2,4-Dinitrophenylhydrazine

To a solution of 0.4 g 2,4-DNPH in 100ml 2N hydrochloric acid, added 1ml ethanol. Aldehydes and ketones give yellow or red spots on spraying the plate with this solution.

Spectroscopy

Nuclear magnetic resonance spectroscopy

The $^1$H-NMR spectra of the isolates were recorded under room temperature on Bruker ARX 500 instrument at 500 MHz in DMSO-$d_6$. TMS was used as the internal standard. The $^{13}$CNR was obtained at 125 MHz.

Mass spectroscopy

The Mass Spectra and the High Resolution Mass Spectra of the compounds were recorded on FINNEGAN MAT 8200 and FISON MD 800 instruments.
2.3.3 Experimental

Extraction

The conical prickles collected were already in the dried form. Coarsely powdered 5Kg of the plant material and extracted successively with 3 X 7L of light petroleum ether and acetone.

The extraction was carried out by boiling the material in respective solvents taken in a round bottom flask fitted with a water condenser, over a water bath. Refluxed the material until the solvent started to boil and the hot content was left standing overnight. Then filtered and collected the extract and added fresh solvent to residue. The process was repeated three times so as to complete the extraction. The extract collected was combined and the total volume was reduced to 200ml by distilling off the solvent under reduced pressure. The concentrated extracts were stored under refrigeration for further study.

Fractionation

The extracts were adsorbed on 250gm silica gel. This was then loaded on a preparative column. (Dimension usually being 7cm X 100cm; d X l) The column was packed with silica gel as the stationary phase which was wetted using light petroleum ether to achieve least polarity to the mobile phase during the beginning of elution. The mobile phase for elution was fixed based on the TLC analysis. The elution was carried out by gradient elution technique; the gradation of the mobile phase polarity was achieved by homogenous mixing of the solvents with different polarity. The different fractions collected were again analysed using TLC and the similar fractions were combined and concentrated under vacuum to 50ml. These fractions were also stored under refrigeration for further analysis.
The Acetone Extract

The concentrated acetone extract after removal of the solvent, yielded 98g of a dark green residue. This was then dissolved in 200ml hot acetone and adsorbed evenly on 250g silica gel. It was then loaded on a preparative column using silica gel wetted with petroleum ether as the stationary phase. Mixture of ethyl acetate and petroleum ether in the ratio 1: 4 gave good band separation in the TLC. Hence a gradient elution starting with 100% petroleum ether to 100% ethyl acetate was chosen as the eluting medium.

The elution of the column was started on with 500ml 100% petroleum ether. It was continued by using 10%, 11% and 12.5% ethyl acetate in sequence. These eluents were similar from TLC analysis and hence combined them and concentrated the total fraction under vacuum to yield a red syrupy liquid (ZAR). The elution of the column was resumed with 14% and 17% ethyl acetate successively. These fractions collected being similar were combined and concentrated to get another syrupy liquid (ZAY) which was yellow in colour. Both ZAY and ZAR were found to be mixtures of large number of compounds and were analysed by GC-MS. This study is detailed in section 2.4.

On eluting the column with 20% ethyl acetate, the fractions obtained showed a bluish florescence in white light. The like fractions were combined and allowed standing for 15 hours. A shiny crystalline white solid (ZX) separated from this dilute solution. Filtered and collected this solid after washing well with the corresponding mobile phase. The filtrate was concentrated to yield more of this solid. ZX dissolved freely in chloroform and TLC showed a single spot with bright blue fluorescence in long UV. A good yield of 800mg of ZX was obtained. This solid melted at 188°C. The polarity of the mobile phase was further increased to 25% ethyl acetate. Many dissimilar fractions containing more than one compound were collected. Out
of this one fraction afforded a white solid in very less quantities and insufficient for detailed analysis. The filtrate in this case was concentrated to 25mL which on keeping undisturbed for 72 hours gave a reddish solid which on recrystallisation from ethanol yielded a single orange red crystal (ZO) that weighed 31.8mg. Its melting point was found to be 180°C. On eluting the column with 33.3% ethyl acetate, once again, many like fractions were procured. Two solids could be isolated from this polarity range. The first compound appeared on concentrating the combined elute as yellow crystalline residue which on recrystallisation from DMSO gave fine bright yellow needle like crystals (ZY) and melted at 288°C. This compound produced a yellow fluorescence in ultraviolet light. Subsequently the second compound was obtained as a white amorphous solid which on recrystallisation from pyridine gave white powdery substance (ZQ) that melted at 268°C and soluble in DMSO. This compound furnished single non fluorescent spot in TLC which turned pink on spraying the plate with 20% H₂SO₄ and heating it to 110°C. The polarity of the mobile phase was further increased to 50% ethyl acetate. A dirty white residue obtained which was recrystallised from pyridine to get 11g of the pure white crystal (ZW) which melted at 313°C. All these compounds gave a positive test with Dragendorff reagent indicating their identity to be nitrogen containing compounds mainly alkaloids. A comprehensive description on the structural elucidation of the above compounds is given in section 2.5.

2.3.4 Synthetic Analogue of ZX

Reduction of the carbonyl group of ZX using sodium borohydride was carried out as shown in scheme 2.3a. For this 102mg of ZX (ca. 0.251mmol) and 29mg of sodium borohydride (ca. 0.766) was taken in 10ml isopropanol and refluxed for six hours. Added four drops of water and continued refluxing for further two hours. After the conversion of the functional group was
observed in TLC as the increase in polarity and the decrease in $R_f$, poured the reaction mixture to 0.5ml concentrated hydrochloric acid to get a white solid. This on recrystallisation from isopropanol: ethanol (1:2) gave 77.2mg (ca. 0.19 mmol) of white crystalline product ZXR. The reduction was confirmed from the spectral analysis which is also given in section 2.5.

![Scheme 2.3a Reduction of ZX to ZXR](image)

**2.3.5 Biological Activity Studies**

The five isolates as well as the synthesized compound ZXR were analysed for their activity against various microbial test organisms in an agar diffusion assay. The organisms used for the study were *Escherichia coli*, *Bacillus megaterium*, *Microbotryum violaceum* and *Chlorella fusca*. 50 μg of test substance/test filter disc (50 μL at a concentration of 1μg/μL) of the pure substances and of the control substances were tested in an agar diffusion assay; radius of zone of inhibition was measured which can be correlated to the activity against the microorganism. Penicillin, Tetracycline, Nystatin and Actidione were used as controls. The cytotoxicity studies of ZX, ZXR and ZQ also were done on seven human cancer cell lines using MTT assay. The detailing of these studies is presented in section 3.
4. GC-MS ANALYSIS THE SYRUPY FRACTIONS FROM ACETONE EXTRACT

The red syrupy fraction (ZAR) was obtained from the column during the fractionation of the acetone extract of the conical prickles on the stem bark of *Z. rhetsa* on elution with 500ml 100% petroleum ether and 10%, 11% and 12.5% ethyl acetate in sequence and the yellow syrupy fraction (ZAY) was obtained on elution with 14% and 17% ethyl acetate successively. On TLC examination of these fractions, large number of compounds was found to be present. Hence they were subjected to GC-MS analysis to identify the components present.

2.4.1 Experimental

For GC/MS measurements a GC-17A with QP5000 (Shimadzu) and Compaq-ProLinea data system (class 5k-software), a GC-HP5890 with HP5970-MSD (Hewlett-Packard, USA) and ChemStation software on a Pentium PC (BoÈhm, Austria), a GCQ (Finnigan-Spectronex, Germany-USA) and Gateway-2000-PS75 data system (Siemens-Nixdorf, Germany, GCQ software) were used. The carrier gas was helium; injector temperature, 250 °C; interface-heating at 300 °C, ion-source heating at 200 °C, EI-mode was 70 eV, and the scan-range was 41± 450 amu. The temperature programme was: 40 °C/5 min to 280 °C/5 min, with a heating rate of 6 °C/min. The columns were 30 m X 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 mm (Biorad, Germany) and 30 m X 0.32 mm bonded Stabilwax, with a film thickness of 0.50 mm (Restek, USA). Quantification was achieved using peak area calculations, and compound identification was partly carried out using correlations between retention times and online Wiley, NBS and NIST library spectra. Both red and yellow fractions were analysed under the same conditions described above.
## Table 2.4a Composition of the red syrupy fraction

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di isopropyl disulphide</td>
<td>1113</td>
<td>0.08</td>
</tr>
<tr>
<td>4-acetyl-1-methylcyclohexene</td>
<td>1135</td>
<td>0.30</td>
</tr>
<tr>
<td>Ditertbutyl disulphide</td>
<td>1158</td>
<td>0.14</td>
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<tr>
<td>N-propyl-sec-butyl disulphide</td>
<td>1166</td>
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</tr>
<tr>
<td>Dodecane</td>
<td>1200</td>
<td>0.54</td>
</tr>
<tr>
<td>Bis-(1-methylpropyl)disulphide</td>
<td>1217</td>
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</tr>
<tr>
<td>Hexylcyclohexane</td>
<td>1241</td>
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<td>2-Undecanone</td>
<td>1293</td>
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<td>Tridecane</td>
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<td>α-Copaene</td>
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<td>Tetradecene</td>
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<td>α-cis Bergamotene</td>
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<td>α-Santalene</td>
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<td>β-Caryophyllene</td>
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<tr>
<td>α-trans Bergamotene</td>
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<tr>
<td>β-epi Santalene</td>
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<td>α-acoradiene</td>
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<td>β-acoradien</td>
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</tr>
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<td>Curcumene ar</td>
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<tr>
<td>Pentadecane</td>
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<tr>
<td>α-cis Bisabolene</td>
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<td>3.49</td>
</tr>
<tr>
<td>β-bisabolene</td>
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<td>β-Sesquiphellandrene</td>
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<tr>
<td>α-trans Bisabolene</td>
<td>1545</td>
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<td>Hexadecene isomer</td>
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<tr>
<td>Decylcyclohexane</td>
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<td>Butylstearate</td>
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<tr>
<td>Fatty acid ester</td>
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<td>13.57</td>
</tr>
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</table>

**Total identified** 75.73
Table 2.4b Composition of the yellow syrupy fraction

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<th>Compounds</th>
<th>RI</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecene</td>
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<tr>
<td>Hexylcyclohexane</td>
<td>1280</td>
<td>0.38</td>
</tr>
<tr>
<td>Tridecane</td>
<td>1295</td>
<td>0.24</td>
</tr>
<tr>
<td>Ditertbutyldisulphide</td>
<td>1339</td>
<td>0.10</td>
</tr>
<tr>
<td>Tetradecane</td>
<td>1398</td>
<td>1.63</td>
</tr>
<tr>
<td>Bis(1-methylpropyl) disulphide</td>
<td>1424</td>
<td>0.88</td>
</tr>
<tr>
<td>Tetradecene</td>
<td>1435</td>
<td>2.39</td>
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<tr>
<td>Octylcyclohexane</td>
<td>1485</td>
<td>0.86</td>
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<tr>
<td>Pentadecane</td>
<td>1499</td>
<td>0.15</td>
</tr>
<tr>
<td>4-acetyl-1-methylcylohexene</td>
<td>1528</td>
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</tr>
<tr>
<td>Cis-α-bergamotene</td>
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</tr>
<tr>
<td>Trans-α-bergamotene</td>
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<tr>
<td>β-Caryophyllene</td>
<td>1576</td>
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<tr>
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<tr>
<td>Cadalene</td>
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<td>0.15</td>
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<tr>
<td>Butylpalmitate</td>
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<td>5.61</td>
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<tr>
<td>Butylstearate</td>
<td>2642</td>
<td>6.51</td>
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<tr>
<td>Fatty acid ester</td>
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<tr>
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<tr>
<td><strong>Total identified</strong></td>
<td></td>
<td><strong>85.89</strong></td>
</tr>
</tbody>
</table>

2.4.2 Results

From the red syrupy fraction, on GC-MS analysis, 42 compounds were identified. β-Bisabolene (5.69%) was found to be the major terpenoid component. Ar curcumene (3.48%), α-Copaene (0.22%), β-trans-farnesene
(0.23%), α-trans-bergamotene (1.08%), α-santalene (0.07%), α and β-acoradiene (0.14 and 0.17%), β-sesquiphellandrene and cadalene (0.79%) were also present in lesser amounts. Apart from the terpenoid components, there were five fatty acid esters in appreciable amounts, comprising of a total of 40.75%. The different compounds identified in ZAR and their percentage compositions are given in Table 2.4a.

β-Bisabolene (9.79/%) is found to be the major terpenoid component in the yellow fraction also. The compounds ar curcumene (3.81%), trans-α-bergamotene (1.28%), β-caryophyllene (0.51%), δ-cadinene (0.24%) and epi-β-santalene (0.25%) were also identified. The yellow fraction contained seven fatty acid esters which sum up to a total of 52.87%. The chemical composition of this fraction is given in Table 2.4b.

2.4.3 Discussion

Many chemical defense reactions are induced in plants by external agents. These reactions are complex and include the release of various stress compounds. For example feeding of the white pine weevil Pissodes strobi on the stem of conifers increases the activity of the enzymes involved in the biosynthesis of terpenes whereby there is the accumulation of terpenes in the tissues resulting in the release of α- and β-farnesenes and linalool1,2. (E)-β-farnesene has insect repellant properties and has been shown to possess potent field activity against aphids, acting as an alarm pheromone for many aphid species. The compounds (E)-Caryophyllene and α-cis bergamotene are also found to be involved in plant defense due to their significant insect repellent activity. It is found that caterpillar feeding on Nicotiana attenuata induced significant increases in whole-plant emission the latter compound3. There is also some evidence that (E)-caryophyllene play an important role in nematode host localization of Zea mays, the third major cereal crop in the world after wheat and rice, and the most important crop in sub-Saharan Africa. Seedlings
of this plant release the \((E)\)-caryophyllene into the soil from their roots in response to feeding by the root attacking herbivore, the western corn root worm, *Diabrotica virgifera*. This compound attracts the entomopathogenic nematode, *Heterorhabditis megidis* that feeds on the herbivore whereby protecting the plant\(^4\). The compound is also found to be cytotoxic and feeding deterrent to some herbivores. So are the monoterpenes, \(\alpha\)-pinene and limonene and the sesquiterpene \(\alpha\)-copaene\(^5\).

The red and yellow syrupy fractions studied here contain most of the above biologically significant compounds and many more. \(\beta\)-Bisabolene, the chief terpene present in both the syrupy fractions may be associated with antiplasmodial properties. The malaria causing *Plasmodium falciparum*, which is increasingly becoming resistant to available antimalarial agents were found to be highly sensitive to the stem bark extract of *Uvariastrum pierreanum* which has \(\alpha\)-bisabolol (11.50%) and \(\beta\)-bisabolene (28.20%) as the major components\(^6\). The presence of such active compounds in spines of *Z. rhetsa* increases its practical applicability. The conical prickles in the raw form itself may be useful in agriculture for the control of pests. This requires field investigations. The exact biological activities of these syrupy fractions can also be further undertaken. Structures of major components are given in appendix.
**REFERENCES**


5. ISOLATION OF ALKALOIDS

This section deals with the isolation of five alkaloids including a new compound from the acetone extract of the stem bark conical prickles of *Z. rhetsa*. The concentrated extract was fractionated using column chromatography. Separation was achieved by means of gradient elution, staring with 100% petroleum ether via an escalation of polarity achieved by homogenous mixing of ethyl acetate with petroleum ether, ending with 100% ethyl acetate and a final wash with methanol. This process yielded five pure crystalline alkaloids whose structures were determined by various spectral analyses which are described below. The structural characterization of the reduction product of one of them is also discussed. The isolation and characterization of the five alkaloids has been published in the Natural Products Communications. (Krohn K, Cludius-Brandt S, Schulz B, Sreelekha M, Shafi PM (2011) Isolation, structure elucidation and biological activity of a new alkaloid from *Zanthoxylum rhetsa*. Natural Products Communications 6(11):1595-1596).

2.5.1 Characterisation of the white crystalline solid, ZX

The compound obtained as a white crystalline solid gave blue fluorescence in UV 366 nm, during elution with 20% ethyl acetate. It gave a positive test with Dragendorff’s reagent and 2,4-dinitrophenylhydrazine and a yellow spot on spraying the plate with 20% aqueous sulphuric acid and heating to 110°C. This result suggests the compound to be an alkaloid with a carbonyl group. The melting point was determined to be 188°C. The optical rotation $[\alpha]_D$ was found to be $-132^\circ$ (c 0.1g, CHCl$_3$)

The molecular mass of the compound was found from the positive HREIMS as 405.14936, indicating a molecular formula of C$_{24}$H$_{23}$NO$_5$ for which the
calculated molecular mass is 405.15761 (the spectrum given is at a resolution of 1000 only). This molecular formula corresponds to 6-acetonyldihydrochelerythrin earlier isolated from different plants\textsuperscript{1-3}. All the spectra were also identical to that reported for 6-Acetonyldihydrochelerythrin\textsuperscript{4}. This conclusion is supported by an intense peak at m/z 347.9885 in the mass spectrum which is due to the formation of chelerythrin by the loss of acetonyl group (–CH$_2$COCH$_3$).

The $^1$H NMR (500 MHz, CDCl$_3$) spectrum has a singlet at $\delta$ 2.06 (3H) corresponding to the three protons of –COCH$_3$. Two singlets, each integrating to three protons are present at $\delta$ values 3.93 and 3.96, corresponding to the two methoxy groups. A two proton singlet at $\delta$ 6.04, characteristic of a methylenedioxy group, is also there in the spectrum. Also there is a three proton singlet at $\delta$ 2.65 corresponding to NCH$_3$ protons. The –CH$_2$– protons adjacent to the carbonyl group appear as a doublet of doublet at $\delta$ 2.26 ($J=14.8$Hz and 3.8Hz) and at $\delta$ 2.59 ($J=14.8$Hz and 11.1Hz). These two protons, although attached to the same carbon atom (HMOC cross peak with carbon at $\delta$ 46.8) are chemically non-equivalent because of the chiral carbon (at C-6) adjacent to it. The proton at C-6 appear at $\delta$ 5.05 as a doublet of doublet ($J=11.3$Hz and 3.8Hz). Two pair of orthocoupling doublets at $\delta$ 6.95 ($J=8.7$Hz), 7.70 ($J=8.7$Hz) and 7.48 ($J=8.4$Hz), 7.53 ($J=8.4$Hz) and two singlets at $\delta$ 7.10 and 7.52 appear in the aromatic region.

$^{13}$C NMR (125 MHz, CDCl$_3$) values for the compound are given by 31.0 (CH$_3$), 42.8 (NCH$_3$), 46.8 (CH$_2$), 54.9 (CH), 55.8 (OCH$_3$), 60.9(OCH$_3$), 100.6(CH), 101.0 (CH$_2$), 104.3 (CH), 111.6 (CH), 118.8(CH), 119.7 (CH), 123.3 (C), 124.8 (C), 127.3 (C), 128.1 (C), 131.0 (C), 139.2 (C), 145.5 (C), 147.6 (C), 148.1 (C), 152.1 (C) and 207.4 (C).

From the above spectral details, this white crystalline compound named ZX is characterised to be 6-acetonyldihydrochelerythrin whose
structure is as given below which is also supported by the COSY, DEPT, HMQC AND HMBC spectra of the compound.

There is a chance that this compound is an artefact form from chelerythrin, which has been reported from this plant, during extraction with acetone as acetone is known to react with chelerythrin to form acetonyldihydrochelerythrin. But on extracting the plant material with methanol alone, in a different experiment to obtain the total alkaloid content of the plant, we could isolate this compound from the extract. This finding combined with the fact that the compound was optically active confirms that it is not an artefact of chelerethryn but originally present in the prickles.
2.5.2 The Characterisation of the orange red crystals, (ZO)

A reddish solid was obtained from the column on concentration of similar fractions on elution with 20% ethyl acetate. This on recrystallisation from ethanol yielded orange red crystals that melted at 180°C. The mass spectrum showed a molecular ion peak at m/z 259.2 and a base peak at 85.1. Odd mass is indicative of odd number of nitrogen in the compound. It gave a positive test with Dragendorff’s reagent, proving its identity to be an alkaloid. Skimmianine, an alkaloid with molecular mass 259 has already been reported from this plant.

The proton NMR spectrum has three singlets at δ values 4.02, 4.11 and 4.42 each integrating to three protons. These correspond to the three methoxy groups. Two doublets at δ 7.22 (J= 9.5Hz) and δ 8.01 (J= 9Hz) correspond to the two ortho coupling aromatic protons. The two protons in the furanoid ring absorbs at δ 7.53 (J= 3Hz) and δ 7.57 (J= 3Hz). The $^{13}$C values 56.8(CH$_3$), 59.2(CH$_3$), 61.7 (CH$_3$), 102.3(CH), 104.6(CH), 112.4(CH), 114.9 (C), 118.1(CH), 141.5(C), 142.1(C), 143.0(CH), 152.1(C), 157.2(C) and 164.3(C) can also be correlated to the structure of skimmianine from the earlier reports. Hence the orange red crystal obtained is inferred to be skimmianine. This inference is supported by the DEPT, $^1$H-$^1$H COSY, HMBC and HMQC exeriments. The structure is given below.
2.5.3 The characterisation of the bright yellow crystals, ZY

The compound was obtained as yellow needle like crystals when recrystallised from DMSO and it melted at 288°C. The compound gave positive test with Dragendorff’s reagent. The EI Mass spectra yielded the positive molecular ion peak which was also the base peak at m/z 315.1. The molecular mass was confirmed by HREIMS that gave the molecular ionization peak at 315.11575 indicating the molecular formula C_{19}H_{13}N_{3}O_{2} for which the calculated mass value is 315.1005. The other major peaks corresponds to the m/z (%) values 284.13(30), 286.1(26), 333.1(20), 167(15) and 140.1(14).

The proton NMR spectrum showed the presence of nine aromatic protons. They include two ortho coupling protons at δ values 8.64 (d, 1H, J= 7.6Hz) and 7.87 (d, 1H J= 7.6Hz) corresponding to H-7 and H-8 respectively, the two doublets and a doublet of doublet at δ values), 7.46 (d, 1H, J=6.0 Hz), 7.94 (dd, 1H, J= 6.0 Hz, 3.6 Hz) and 7.44(d, 1H, J=3.6 Hz) corresponding to H-2, H-3 and H-4 protons respectively and four mutually coupling protons at δ values 7.77 (d, 1H, J= 7.3Hz ), 7.51(t, 1H, J= 7.2Hz) , 7.31(t, 1H, J= 7.3Hz) and 8.21 (d, 1H, J= 7.3Hz) due to H-9, H-10, H-11 and H-12protons respectively. In addition the presence of a methoxy and an NH group was revealed by signals at δ 4.04 and a broad singlet at δ 12.5 (1H, br s, exchangeable with D_{2}O), respectively.

^{13}C NMR signals were obtained at δ 56.4 (OCH_{3}), 108.8(CH), 113.5(CH), 114.9(CH), 117.3(C), 118.1(CH), 118.4(C), 120.2(C), 121.0(CH), 121.2(CH), 122.3(C), 125.2(CH), 127.0(CH), 130.1(C), 139.3(C), 139.5(C), 140.5(C), 154.4(C), 158.9(C).

These spectral data correspond to 7,8-dehydro-1-methoxyrutaecarpine, earlier isolated from Z. integrifoliolum. This finding was further confirmed
by $^1$H-$^1$H COSY, HMBC and HMQC and DEPT experiments. The structure of the compound is as given below.
2.5.4 The characterisation of the white amorphous powder, ZQ

The compound ZQ showed no significant fluorescence under UV on silica gel TLC plates but gave positive reactions with Dragendorff’s reagent. It was isolated as a white amorphous powder that melted at 268°C. Its HREIMS was obtained at m/z 381.12088 corresponding to the molecular formula C$_{21}$H$_{19}$NO$_6$ for which the calculated molecular mass is 381.12123. The odd mass and the reaction with Dragendorff’s reagent indicate the compound to be an alkaloid.

The $^1$H NMR spectrum displayed, in the aromatic region, two singlets at δ values 7.08 and 7.19 corresponding to H-1 and H-4. There is also two pair of ortho-coupling doublets at δ values 7.72 and 7.31 corresponding to H-5 and H-6 and δ value 6.54 and 6.80 corresponding to H-11 and H-12 respectively in this region. The spectrum gave two methoxyl group protons at δ 3.90 and 3.92 and exhibited a singlet integrating to two protons at δ 6.08 typical of a methylenedioxy group signal. Moreover, the $^1$H NMR spectrum also showed the three proton singlet at δ 3.01 and one proton singlet at δ 8.16 which can be attributed to N-methyl formamide of which the corresponding carbons appeared at 33.2 and 164.5 on $^{13}$C NMR spectrum. The rest of the signals in $^{13}$C NMR spectrum (125 MHz, DMSO-d$_6$) for the compound are 55.9 (OCH$_3$), 61.3 (OCH$_3$), 99.3 (CH), 101.5(CH$_2$), 104.1 (CH), 104.3 (CH), 118.6 (C), 125.0(CH), 127.4(CH), 127.5 (CH), 128.8 (C), 131.3 (C), 133.4 (C), 135.6 (C), 135.8(C), 146.7(C), 148.1(C ),149.3 (C) and 152.0 (C).

The above evidences combined with the HMBC, HMQC and DEPT spectra of the compound and comparison of the data from the earlier report proves the compound to be arnottianamide$^{8,9}$. This is the first report of this compound from *Z. Rhetsa*. The structure of the compound is as given below.
2.5.5 The new quinazoline alkaloid from the plant ZW.

Compound ZW was isolated as a white powder which was recrystallised from pyridine as white crystals. The compound gave a positive test with Dragendorff’s reagent indicating the compound to be an alkaloid. The melting point of the compound was 313°C. The HREIMS a pseudomolecular ion peak at \( m/z \) 279.02437 \([M+H]^+\) that corresponds with the molecular formula \( \text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_3 \) for which the calculated molecular mass is 278.06914.

The 1H NMR spectrum, in combination with the data from the COSY experiment, revealed the presence of two ortho-disubstituted benzene rings, one at \( \delta \) 7.59 \((1\text{H}, \text{t}, J = 7.1 \text{ Hz}, \text{H-8})\), 7.85 \((1\text{H}, \text{d}, J = 8.3 \text{ Hz}, \text{H-10})\), 7.91 \((1\text{H}, \text{t}, J = 6.9 \text{ Hz}, \text{H-9})\), and 8.17-8.19 \((1\text{H}, \text{m}, \text{H-7})\), and the other at \( \delta \) 7.28 \((1\text{H}, \text{t}, J = 7.2 \text{ Hz}, \text{H-2})\), 7.37 \((1\text{H}, \text{t}, J = 7.2 \text{ Hz}, \text{H-3})\), 7.71 \((1\text{H}, \text{d}, J = 8.2 \text{ Hz}, \text{H-4})\), and 8.19-8.21 \((1\text{H}, \text{m}, \text{H-1})\). The proton H-1 showed HMBC correlation with an amido carbon (C-12, \( \delta \) 161.4). A singlet at \( \delta \) 12.79 displayed no HMQC but NOE correlation with H-4, indicating the existence of a hydroquinazolinone unit in compound ZW. H-7 correlated with the olefinic carbon C-6 \((\delta \) 108.1), indicating the presence of an indole unit. A broad singlet at \( \delta \) 14.53 \((1\text{H}, \text{s}, \text{COOH})\) was typical for the presence of a carboxyl group \((\delta \) 169.8) attached to C-6. Therefore, in combination with the COSY, HMQC and HMBC spectra, the structure of compound ZW was determined as 5,12-dihydro-12-oxoindolo[2,1-b]quinazoline-6-carboxylic acid, as shown below.
2.5.6 The Sodium Borohydride Reduction Product of ZX, ZXR

The reduction of ZX with NaBH₄ was performed affording the racemic alcohol ZXR as a white solid. This hitherto unknown compound melted at 210°C and furnished a molecular ion peak at m/z 407.04189 (the attached spectrum is of resolution 1000 only) corresponding to the molecular formula C₂₄H₂₅NO₅ (calculated 407.17326). ¹H NMR (500 MHz, CDCl₃) given below was very similar to ZX and the δ value 4.02-4.10 (1H, m, -C(OH)H-) confirms the reduction.

¹H NMR (500 MHz, CDCl₃): 1.01 (3H, d J = 6.3 Hz, Me), 1.38- 1.62 (2H, m, CH₂), 2.69 (3H, s, NCH₃), 3.93 (3H, s, OMe), 3.95 (3H, s, OMe), 4.02- 4.10 (1H, m, -C(OH)H-), 4.61- 4.85 (1H, m, -CH₂CH₂-), 6.05 (2H, s, -OCH₂O), 6.95 (1H, d, J = 8.5 Hz, Ar), 7.11 (1H, s, Ar), 7.47- 7.54 (3H, m, Ar), 7.70 (1H, d, J = 8.6 Hz, Ar).

¹³C NMR (125 MHz, CDCl₃): 23.2 (CH₃), 41.5 (CH₂), 42.9 (NCH₃), 55.9 (CH₃), 58.5 (CH), 61.2 (OCH₃), 68.0 (CH), 99.6 (CH), 101.1 (CH₂), 104.6 (CH), 111.8 (CH), 119.2 (CH), 119.7 (CH), 123.7 (C), 123.9 (CH), 124.7 (C), 127.3 (C), 128.1 (C), 131.2 (C), 139.2 (C), 145.5 (C), 147.7 (C), 148.7 (C), 152.2 (C).

The structure of the compound is as follows.
2.5.7 Biological significance of the compounds

Of all the plant species identified on Earth, relatively a small portion alone is used to satisfy the basic needs of mankind as source of carbohydrates, proteins and fats and other raw materials for food and shelter. Even more are exploited for medicinal purposes and many of the pharmaceuticals of today are based on plant-derived chemicals.

In this section we have isolated four known alkaloids 6-acetonyldihydrochelerythrine, skimmianine, 7,8-dehydro-1-methoxyrutaecarpine and arnottianamide and a new one, 5,12-dihydro-12-oxoindolo[2,1-b]quinazoline-6-carboxylic acid from the conical prickles on the stem bark of *Z. rhetsa*. The sodium borohydride reduction was carried out on 6-acetonyldihydrochelerythrine to obtain yet another new semisynthetic alkaloid, 6-(2-Hydroxypropyl)-dihydrochelerythrine. The alkaloids in general are renowned for their biological activity. Morphine and codeine for instance, are potent analgesics that have been exploited for thousands of years and are produced exclusively in the opium poppy (*Papaver somniferum*). Other alkaloids used as pharmaceuticals include sanguinarine and berberine (antimicrobial), noscapine (antitussive and potentially antineoplastic), papaverine (vasodilator), and (+)-tubocurarine (muscle relaxant). Thebaine is a metabolic precursor to morphine and codeine and is used for the synthesis of analgesics such as oxycodone, naltrexone, and buprenorphine. Most of these compounds are not feasible targets for *de novo* chemical synthesis owing primarily to the occurrence of multiple chiral centers; therefore, plants remain the only commercial sources for many pharmaceutical alkaloids.

There are similar reports on the alkaloids isolated in this study as well. The furoquinoline alkaloid skimmianine, which was obtained in appreciable amounts from the plant, showed invitro anti viral effect against hepatitis B virus and has strong acetylcholinesterase inhibiting activity which is
relevant to the treatment of Alzheimer’s disease\textsuperscript{12}. Skimmianine was found to be cytotoxic against human ovarian cancer cell line\textsuperscript{13} and exhibited inhibition (IC\textsubscript{50} \leq 18.19\mu M) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasinB (FMLP/CB). In addition, it inhibited FMLP/CB-induced elastase release with an IC\textsubscript{50} value of 19.15 \pm 0.66\mu M\textsuperscript{14}. Skimmianine was found to have antiplatelet aggregation activity\textsuperscript{15}. It markedly inhibited the histamine release rat mast cells (RBL-2H3 cells) by mechanisms related to intercellular Ca\textsuperscript{2+} signalling and protein kinase C signalling\textsuperscript{16}. The compound also possessed antitrypanosomal\textsuperscript{17} and leshmanicidal activity\textsuperscript{18} and acted as an antidiarrhoea agent\textsuperscript{19}. Skimmianine was found to have insecticidal effects. It was found to have significant feeding deterrence against two stored product insects, \textit{Tribolium castaneum} and \textit{Sitophilus zeamais}\textsuperscript{20}.

The compounds 7,8-dehydro-1-methoxyrutaecarpine and 6-acetonyldihydrochelerythrine also exhibited cytotoxicities (ED\textsubscript{50} values < 4\mu g/mL) against P-388 and HT-29 cell lines in vitro\textsuperscript{7}. The antimicrobial activities of all the compounds isolated will be discussed in the next section where all of them are active against one or the other organisms.
REFERENCES


