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1. Use of medicinal plants is as old as human civilization and continuous efforts are being made to improve medicinal plants or produce their products in high amounts through various technologies. About 200,000 natural products of plant origin are known and many more are being identified from higher plants and micro-organisms. In spite of various considerable advances taking place in pharmaceutical field especially in the synthetics, plants and their derivatives have been able to maintain their position. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily available at affordable prices and sometime the only source of health care available to the poor.

2. The object of the present study is to explore the medicinal potentialities of two plants, viz. *Azima tetracantha* Lam. and *Cocculus hirsutus* L. *Azima tetracantha* belongs to the family of Salvadoraceae is grown as hedgerows small shrubs. It is a xerophytic plant found in India, Africa, Madagascar and Ceylon. In traditional systems, the root bark is used in muscular rheumatism. The leaves are used as stimulant and are given to puerperal woman immediately after confinement. The leaves are also used for rheumatism and their juice used to relive cough; as diuretic and in treating chronic diarrhea. Further, the leaves mixed with stem bark and made into a semisolid liquid are given to cattle for a week in rinderpest. It has been also reported that the leaf powder is used in toothache. The plant proved to have diuretic effect in rats; powder of leaf possess anti-inflammatory, anti-fungal and antiulcer activities.
Cocculus hirsutus L. is a scandent shrubby creeper and belongs to the family Menispermaceae. It is found growing in tropical and subtropical parts of India from the foot of Himalayas to south India, Ceylon, China, Arabia and tropical Africa. The juice of the leaves is used externally as cooling and soothing application in pruriga (skin itching), eczema (scabies), impetigo (skin eruption) etc. It is used in the treatment of gonorrhea; the decoction of root is given in chronic rheumatism, stomachache, dyspepsia and syphilitic cachexia. It is used in tribal medicine for treating leucorrhoea and menorrhagia. The roots are bitter, laxative, alterant, digestive, carminative, diuretic, aphrodisiac, expectorant, antipyretic and tonic. It is useful in poisonous bites, leprosy, skin diseases, pruritus dyspepsia flatulence, colic, strangury (painful urination), and gout, etc. The plant has been subjected to several pharmacological screening viz. hypoglycemic and cardiotonic activities in roots; diuretic, laxative activities; anti-inflammatory, analgesic and antimicrobial activities.

It is evident that the plants are used for various ailments in folklore system and such medicinal claims of the plants are largely remain unexplored warranting systematic pharmacological evaluation. Further, phytochemical investigations documented the rich source of alkaloids and other important secondary metabolites which attracts any investigator to exploit the plants for rigorous screening for pharmacological activities. Therefore, in the present research programme the two plants are subjected to experimentation for following studies viz. pharmacognosy, phytochemistry, pharmacological screening for hepatoprotective, antioxidant, antipyretic, anti-inflammatory, analgesic, cardiovascular, anthelmintic and antimicrobial activities.
3. The leaves of both plants, *Azima tetracantha* and *Cocculus hirsutus* were collected in regions around Chitradurga, Chitradurga (D), Karnataka in natural condition and subjected to Pharmacognostic study viz. macroscopical, microscopical, stomatal index, vein islet number, vein termination number, palisade ratio and proximate values like extractive values, total ash value and acid insoluble ash value parameters.

The leaves of *Azima tetracantha* are petiolate, entire and elliptic to oblong in shape. The surface is smooth leathery, sharp-tipped or spiny at the apex and are about 4.86 long to 3.31 wide. The stem of the plant is low spinous highly branched bush, woody below but with green herbaceous, almost quadrangular. The microscopic examination transverse section of the leaf having upper and lower epidermis with cuticle, the upper epidermis is multilayered showing the xerophytic character. The stomata are hypostomatic and actinocytic type of cellular arrangements are seen. The mesophyll has upper palisade and lower spongy parenchyma. The microscopy of stem shows outer single layered epidermis followed by outer cortex having parenchymatous starch sheath and sclerenchymatous cells with bundle sheath. Cambium breaks with number of strips. Pith at the center is parenchymatous. Leaf surface data were found to show stomatal index of 11.53, stomatal number 08.70, palisade ratio 04.48, vein islet number 04.30 and vein termination number 04.40. The leaf powder extractive values of petroleum ether, benzene, chloroform, alcoholic and aqueous were 1.55, 1.25, 1.15, 9.95 and 10.5% respectively. The total ash value, acid insoluble ash value and water-soluble ash value was 8.2, 0.16 and 2.09% respectively.

Similarly *Cocculus hirsutus* leaves are alternate, dorsiventral, petiolate, extuplate, ovate, ovate to oblong or sub lanceolate and base
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varies from truncate to cordate. Apex is pointed, mucronate or rarely blunt. Margin is entire, rarely slightly wavy. Both the surfaces of the lamina are tomentose and bear small grey anteriorly directed trichomes, size ranging from 1.2 to 8.8 cm in length and 0.8 to 4 cm in breadth. The microscopic examination of transverse section shows the epidermal cells appear rectangular and are filled with chloroplast and some crystalline substances. Palisade and spongy parenchyma form mesophyll. Cells of spongy parenchyma are thin walled and vary between elongated to rounded forms. Distributed amongst them are certain elongated excreatory sacs filled with brown substances. The vascular bundles of larger veins are enclosed in parenchymatous bundle sheaths. Outer cells of this sheath tend to become sclerenchymatous. The stomata are hypostomatic and anomocytic (ranunculaceous) type of cellular arrangements is seen. The leaf surface data are stomatal index 07.15; stomatal number 05.60, palisade ratio 08.85, vein islet number 04.40 and vein termination number 02.70. Powders of leaf showed extractive values of 1.26, 1.08, 2.30, 10.08 and 6.35 for petroleum ether, benzene, chloroform, alcoholic and aqueous extracts respectively. The total ash value, acid insoluble ash value and water-soluble ash value was found to be 7.90, 0.75 and 1.01 respectively.

4. The powdered leaves of both the plants were collected and extracted with different solvents viz. petroleum ether, chloroform, ethanol and water (Aqueous) respectively. Yield in case of Azima tetracantha, was 1.55, 1.15, 9.95 and 10.5. for pet ether, chloroform, alcoholic and water respectively. Similarly Cocculus hirsutus yielded 1.26, 2.3, 10.08 and 6.35% of dry extracts respectively. All the dried extracts were subjected to qualitative chemical tests and results were tabulated to identify different groups of compounds.
5. Phytochemical analysis of petroleum ether extract of both *Azima tetracantha* and *Cocculus hirsutus* gave positive results for steroids, triterpenoids and tannins. Triterpenoids and steroids were found to be present in chloroform extract of *Azima tetracantha* while, *Cocculus hirsutus* has shown the presence of additional alkaloids. Ethanol extract of *Azima tetracantha* and *Cocculus hirsutus* revealed the presence of carbohydrates, tannins, triterpenoids, steroids, saponins, flavonoids and alkaloids. Carbohydrates, saponins and tannins were found in aqueous extract of both the plants.

6. Since the qualitative analysis revealed the presence of several important secondary metabolites in the alcoholic extract, further isolation of compounds and pharmacological screening has been carried out with alcoholic extract. The residue was dissolved in alcohol and adsorbed on silica gel powder and loaded on a silica gel column. The column was eluted first with n-hexane followed by graded mixtures of n-hexane : ethyl acetate, ethyl acetate and then by graded mixtures of ethyl acetate : methanol. The elution was monitored by TLC. Eluted compounds of *Azima tetracantha* were coded as 1, 2, 3, 4 and 5 and *Cocculus hirsutus* A, B, C and D. The isolated compounds were further subjected to melting point, Mass, IR and Proton NMR spectral analysis for structural elucidation.

The column chromatography performed utilizing alcoholic extract of *Azima tetracantha* resulted in isolation of compounds viz. beta sitosterol, fridelin, 16 beta-hydroxybetulinic acid, taraxerol and rutin by spectral analyses like Mass, IR, and H NMR. Taraxerol, 16 beta-hydroxybetulinic acid and rutin are reported for the first time in this plant. Column chromatography performed for *Cocculus hirsutus* yielded stigmasterol, alpha amyrin, Urs-12-ene-3 beta,22 beta-diol and Beta sitosterol glycoside have
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been eluted and confirmed by chemical tests and Spectral studies. These compounds have been identified forms the first report in the plant.

7. The alcoholic leaf extracts of both plants were subjected to various pharmacological screening. Albino Wistar male rats weighing 150-200g was used for the present study. They were maintained in the animal house of Soniya Education Trust’s College of Pharmacy, Dharwad for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 12 h light-dark cycles. They had free access to standard pellets as basal diet and water ad libitum. All the studies conducted was approved by the Institutional Animal Ethical Committee (IAEC) of SET’s College of Pharmacy (Registration NO.SETCP/IEAC/2008-2009/0544), Dharwad, Karnataka, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Animals weighing between 125-150 g were used for the acute toxicity study and were divided into groups of 6 animals each. The test extracts was administered orally as a suspension in Tween 80 to the different groups in increasing dose levels of 10, 40, 100, 400, 1000, 2000 and 5000mg/kg body weight. 1/20th and 1/10th weight of maximum tolerated dose i.e. 250 and 500 mg /kg body was chosen as effective doses for the study.

8. Hepatoprotective activity was studied by CCl4 induced rat liver damage model. Rats were divided into 7 groups, with six animals in each group. Group I was categorized as normal control and was administered, a single daily oral dose of vehicle for 12 days. Group II was inducer control, were given with a single daily dose of vehicle for 12 days. Group III to VI animals were treated with alcoholic extracts of Azima tetracantha and Cocculus hirsutus at doses of 250 and 500 mg/kg body weight respectively for 12 days.
Group VII animals were administered with standard Silymarin. On 13th day hepatic injury was induced to the animals (all the groups except group I) through oral administration of CCl₄ diluted with liquid paraffin oil (1:1) at a dose of 1 ml/kg body wt for a day. The blood was drawn through retro-orbital plexus of rats on 14th day and subjected to blood serum analysis. Animals were sacrificed and livers were collected to study histopathological and antioxidant parameters. The following methods were employed to establish hepatoprotective activity. Blood serum analysis was carried out to estimate the liver function markers viz. total protein, albumin, total bilirubin, direct bilirubin, Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT).

Serum levels of SGPT (ALT), SGOT (AST), total protein, albumin, total and direct bilirubin in normal and CCl₄ treated rats was studied on animals treated with single oral dose of CCl₄ (1ml/kg; p.o), developed significant liver damage as evident from significant increase in serum activities of SGPT (ALT), SGOT (AST), total & direct bilirubin concentrations and decrease in total protein and serum albumin compared to normal control rats. Oral administration of reference standard silymarin (100 mg/ kg) and various test extracts of *Azima tetracantha* and *Cocculus hirsutus* at 250 and 500 mg/kg daily for 12 days on 13th day CCl₄ treated rats exhibited significant reduction in serum activities of SGPT, SGOT, total and direct bilirubin concentration, whereas significant increase in serum albumin and total protein concentration compared to CCl₄ treated group. Alcoholic extracts of both plants at 500 mg / Kg body weight have considerably protected the toxic effects of CCl₄ on liver.

The liver of normal and treated animals were sectioned and stained with haematoxylin and eosin. Degeneration and necrotic damage produced by CCl₄ was observed microscopically. In liver sections of both the plant extracts
treated rats showed a marked protection in CCl₄ induced liver damage with evident reduction in necrosis and fatty infiltration compared to CCl₄ treated group.

9. Antioxidant activity study was evaluated by enzymatic and non enzymatic parameters. The liver was perfused with cold saline 0.1M phosphate buffer, cut into small pieces and required quantity of liver was weighed and homogenized using ice-cold saline-EDTA in a glass homogenizer. The homogenate was used to estimate non enzymatic antioxidants like total protein, lipid peroxidation, glutathione, and total thiols . The enzymatic antioxidant evaluation was performed by catalase assay.

In vivo liver markers viz. reduced glutathione (GSH), total thiols and catalase activity are significantly reduced in the CCl₄ damaged rats where as the MDA level is significantly increased. However the rats treated with Azima tetracantha extracts at both doses and Silymarin has shown the significant increase in GSH, total thiol and catalase activity, while MDA level was reduced at similar doses. Similarly extracts of Cocculus hirsutus L. at both doses has shown elevation in levels of GSH, total thiol and catalase and diminution in MDA level.

10. Antipyretic activity study was carried out by yeast induced pyrexia in rats. The rats were divided in to 6 groups of 6 animals each. Group I served as normal control and group II as standard Group III to VI received alcoholic extracts of Azima tetracantha and Cocculus hirsutus at two different concentrations viz. 250 and 500mg for each extract respectively. After measuring the basal rectal temperature, all the animals were given a subcutaneous injection of 10 ml/kg body weight of 15% w/v yeast suspended in saline solution. After 18th hour of yeast injection, the animals were again
restrained in individual cages for recording of their rectal temperatures as described previously. Eighteenth hour after injection, the vehicle, test extracts and Paracetamol are given orally. Rats were restrained for recording the rectal temperature from 18th hour at 1 hour intervals up to 22 hour after yeast injection and the results were tabulated. A rise in temperature was recorded from 18th h after yeast injection at 19, 20, 21 and 22nd h in yeast induced group of rats. The effect of alcoholic extracts of *Azima tetracantha* at 250 mg dose showed significant reduction in pyrexia at 21 and 22 h. While, at 500 mg the pyrexia has reduced significantly (P<0.01) at all the experimental intervals indicating dose efficiency. Similarly alcoholic extracts of *Cocculus hirsutus* have also shown significant reduction in pyrexia at 250 mg dose at 20, 21 and 22nd h of rectal temperatures. In higher dose of 500 mg, the temperature reduced at 19, 20, 21 and 22nd h (P<0.01) and the effects are comparable to the standard drug Paracetamol.

11. Carrageenan induced rat paw edema was used to study anti-inflammatory activity using plethysmograph apparatus. The rats were divided into 6 groups of 6 animals each, group I received only vehicle while group II served as standard. Group III to VI was treated with alcoholic extracts of *Azima tetracantha* and *Cocculus hirsutus* at doses of 250 and 500 mg for each extract. Edema was induced by injecting 0.1 ml of 1% carrageenan suspension in the plantar tissue of the paw of all the animals. The relative increase in paw volume was measured in control, standard and extracts treated groups at 30, 60, 120 and 180 min after carrageenan injection.

In the present study, Administration of carrageenan to the control group of rats showed a rise in paw volume at different time intervals viz. 1, 2 and 3 h. However oral administration of alcoholic extracts of *Azima tetracantha* at 250 mg dose exhibited significant reduction in inflammation
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induced by carrageenan at 1h 2h and 3h manifesting a percent reduction of 26.92, 40.62 and 44.00. The reduction in paw volume was more prominent at 500 mg dose by recording mean values and percent inhibition of 0.34±0.01 (34.61%), 0.34±0.03 (46.87%) and 0.24±0.04 ml (52.00%) at 1, 2 and 3h respectively. Similarly alcoholic extracts of Cocculus hirsutus also exhibited a significant (P<0.01) dose dependent anti inflammatory activity by reducing the paw volume. Further, the percentage of inhibition was found to be 37.50 and 30.00% at 250 mg and 26.92, 43.75 and 48.00 at 500 mg dosage. It is also evident from the data that the anti-inflammatory activity was positively correlated to time intervals exhibiting highest inhibition of paw volume at 3 h at all the treatment under study.

12. Analgesic activity was carried out by tail flick method. The basal reaction time to radiant heat source was taken by placing the tip of the tail on the radiant heat source. The tail with drawl from the heat is taken as the end point. The rats were divided into 6 groups of 6 animals each, group I served as normal control and II as standard. Group III to VI received alcoholic extracts of Azima tetracantha and Cocculus hirsutus at two concentrations viz. 250 and 500mg for each extract respectively. The activity was compared between normal control, extracts of Azima tetracantha, Cocculus hirsutus and standard. Percentage of increase in analgesic activity was noted. In the present investigation for analgesic effect of alcoholic extracts of Azima tetracantha on tail flicking in rats at different time intervals viz. 0, 30, 60, 90 and 120 min, rats treated with alcoholic extracts at 250 and 500 mg/kg dose recorded significant (P<0.01) increase over the control in reaction time while, alcoholic extracts of Cocculus hirsutus at 250 mg concentration elevated the reaction time significantly (P<0.01) at 90 and 120 min only whereas, at 500 mg dosage the reaction time was significant at all the different time intervals indicating dose dependent analgesic effect. It is
interesting to note that the activities of both the plant extracts are more effective in later phases of treatment. Further, the *Azima tetracantha* has more effective analgesic property compared to *Cocculus hirsutus*. Notably the alcoholic extract of the latter @ 500mg/kg at 120 min has recorded higher reaction time than the paracetamol indicating its analgesic potential.

13. Cardiovascular activity was studied by Langendorff’s heart preparation model in rats. Rat’s heart was perfused retrogradely with a Krebs-Henseleit solution (pH 7.4). The administration of single dose of 0.2ml alcoholic extract of *Azima tetracantha* (1mg/ml) induced significant (P<0.01) decrease in heart rate to 80.50 (26.8% of negative chronotropic effect) from normal 110.00 BPM and force of contraction was increased to 11.25 mm (55% positive inotropic effect) from 7.25 (normal), whereas Coronary Perfusion Rate (CPR) was decreased (9.12 ± 0.08 ml/min) from initial 14.08 ±0.22 ml/min to an extent of 35.22% . Further increase in dose to 0.6 ml after achieving normal heart rate decreased the heart rate to 41.00 (62.72% of negative chronotropic effect) but increased the force of contraction to 13.00 mm. (79.31% positive inotropic effect)while, CPR was reduced to 5.35 ml/min to an extent of 62.00% indicating dose dependent cardiotonic activity.

Similarly, alcoholic extract of *Cocculus hirsutus* at 0.2 and 0.6ml doses reduced the heart rate (78.50 and 40.75 BPM) reducing by 28.6 and 62.9% of negative chronotropic effect; coronary flow rate was reduced to 8.27ml and 5.18 ml/min reducing by 41% and 63.2%; increase in force of contraction was noted at 12.25 ± mm and 15.00 showing an elevation to the extent of 68.96 and 106 % of positive inotropic effect respectively. The data proves that both the plants *Azima tetracantha* and *Cocculus hirsutus* posses negative chronotropic and positive inotropic effect on isolated rats’ heart showing significant results in a dose dependent manner. A close observation of the
data reveals the superiority of the plant *Cocculus hirsutus* for cardiotonic features as it has shown better mean values for the parameters under study.

14. Anthelmintic activity was studied on *Pheretima posthuma* and *Ascaris galli* separately by *in vitro* method. Six worms of equal length were placed in petri-dish for each concentration separately. Suspension of the extracts (0.1, 0.5 and 1%) and standard drug Albendazole citrate were prepared in 1% acacia solution and poured in to the petridish. A control was also maintained in 1% acacia in saline. The extracts were evaluated for the time taken for complete paralysis and death of earthworms and fowl-round worms. Among the extracts of *Azima tetracantha* and *Cocculus hirsutus* pet ether has shown the maximum reduction in time for paralysis, followed by alcohol, chloroform and aqueous. Similarly, in regard to the time taken to death (minutes), among the extracts, pet ether has shown the maximum reduction in time followed by alcohol, chloroform. Aqueous extract did not show significant activity for this parameter vs. standard at any of the concentration tested. Comparison of the results on the effect of different extracts of leaves of the two plants *Azima tetracantha* and *Cocculus hirsutus* for anthelmintic activity reveals that petroleum ether and alcoholic extracts of both plants are more potent than other extracts. The result indicates a negative correlation between time and concentration of the extracts. Further, among the two plants under study alcoholic extract of *Cocculus hirsutus* is found to be more potent than *Azima tetracantha* irrespective of type of extract, dosage and worms for both the parameters of the activity.

15. Antimicrobial activity study was carried out using the extracts prepared in the course of present research programme were screened for antimicrobial activity against the following gram-positive bacteria *viz.* *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and gram-
negative bacteria viz. Klebsiella pneumoniae and Escherichia coli. For antifungal activity Candida albicans and Aspergillus niger were utilized. The antibacterial activity tests were performed by cup plate method. The extracts were dissolved in minimum quantity of DMSO and adjusted, to make up the volume with distilled water to get 50 and 100μg/ml concentration of extracts. The procaine penicillin, streptomycin and griseofulvin were used as standard for comparison against gram positive, gram negative and for fungal screening respectively. All the solutions were loaded into the well of 8 mm diameter on nutrient agar media and Potato – dextrose agar medium in petriplates for antibacterial and antifungal screening respectively. After incubation, the zone of inhibition in mm was measured and tabulated for comparing the results. In the present investigation among the various extracts viz. petroleum ether, chloroform, alcoholic and aqueous extracts of Azima tetracantha and Cocculus hirsutus, only chloroform and alcoholic extracts showed encouraging results. Both the chloroform and alcoholic extracts exhibited potent antibacterial activity in a concentration dependent manner against the test organisms at concentrations of 50 and 100 μg/ml and are comparable with the standard drug Streptomycin and Procaine penicillin. Both the test extracts have exhibited broad spectrum efficacy by recording remarkable zone of inhibition against all the tested bacteria viz. Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia and E. coli. Among the two extracts studied, alcoholic extract was found to be more effective in terms of antibacterial activity. It was also evident from the results that among the two extracts, alcohol was better in terms of antibacterial activity. The sensitivity order of bacteria in general was found to be Pseudomonas aeruginosa > Escherichia coli > Staphylococcus aureus > Bacillus subtilis > Klebsiella pneumonia. Further, among the two plants Cocculus hirsutus has proved
higher mean values for clear zone of inhibition indicating potential antibacterial property.

The screening of various plant extracts of *Azima tetracantha* and *Cocculus hirsutus* for their plausible antifungal effects against test organisms viz. *Candida albicans* and *Aspergillus niger* were noticed only in alcoholic extract of the several tested in *Azima tetracantha* while chloroform and alcoholic extracts of *Cocculus hirsutus* have shown zone of inhibition in a dose dependent manner indicating the variation in the quality and quantity of phytoconstituents. *Candida albicans* exhibited 12 and 14 mm at 50 and 100 µg/ml respectively, while *Aspergillus niger* has shown 12 and 16 mm of clear zone at similar concentrations. However in *Cocculus hirsutus*, even though, chloroform extract has inhibited the growth of *Candida albicans* and *Aspergillus niger* at 50 and 100 µg/ml concentrations the alcoholic extract recorded higher inhibition against same organisms indicating better efficacy of the latter. Further, the *Candida albicans* was found comparatively resistant than *Aspergillus niger* by virtue of their lesser zone of inhibition. It can be also inferred that the *Cocculus hirsutus* is marginally better for its antifungal activity.

The present scientific studies on the plants *Azima tetracantha* and *Cocculus hirsutus* establishes the potential medicinal uses and justifies some of their ethano-medicinal claims of many folklore systems. The phytochemical investigation in the plants lead to the isolation of compounds viz. 16 beta-hydroxybetalin acid, taraxerol, rutin, stigmasterol, alpha amyrin, Urs-12-ene-3 beta,22 beta-diol and Beta sitosterol glycoside which are reported for the first time in these plants. The various pharmacological screening like hepatoprotective, cardiovascular, anti-inflammatory, antipyretic, analgesic, antimicrobial and anthelmintic activities reveal the
presence of important secondary metabolites of pharmaceutical importance in these plants which could therefore form the basis for the development of new drugs.

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