Chapter 3

Bioprospecting Rutin in Edible Plants of North East India
3.1. Introduction

The traditional medicinal plants are now under extensive studies for revaluation as well as for their therapeutic principles all over the world. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) are involved in a number of diseases\textsuperscript{1,2}. As plants produce a lot of antioxidants to control the oxidative stress caused by solar radiation and oxygen, they can represent a source of new compounds with antioxidant activity.

Rutin is a natural bioflavonoid, listed in the US Pharmacopoeia (USP). Rutin (also called rubuside, quercetin-3-O-rutinoside and sophorin) is a glycoside between the flavonol quercetin and the disaccharide rutinose (\(\alpha\)-L-Rhamnopyranosyl- (1\(\rightarrow\)6)-\(\beta\)-D-glucopyranose). Its name comes from the name of Ruta graveolens, a plant that contain rutin\textsuperscript{3}. It is sometimes referred to as vitamin P, although not strictly a vitamin.\textsuperscript{3}

Rutin (quercetin-3-rutinoside) is a flavonol glycoside that is synthesised by higher plants in defence against ultraviolet radiation and diseases\textsuperscript{4}. Rutin as a secondary metabolite of plants is used for the cure of increased frangibility and permeability of blood capillaries caused by various diseases (vascular-based pathological haemophilia, lesions in the retina in diabetes, in vitamin C deficiency). Other indications are disorders of the function of veins of the lower extremities, their swelling, atherosclerosis or haemorrhoids. Its capacity to intercept free radicals, i.e. to act as an antioxidant, is also very important.
Buckwheat is the main source of rutin; its stems and leaves and seeds containing rutin were categorised as food supplements.

3.2. Occurrences

The major sources of rutin for medical use include buckwheat, fruits and flowers of Japanese pagoda tree, *Eucalyptus macrorhyncha*, the leaves and petioles of Rheum species and Asparagus. Rutin also found in the fruit of the fava d’anta tree (from Brazil), fruits and fruit rinds (especially citrus fruits orange, grapefruit, lemon, lime) and berries such as mulberry, ash tree fruits and cranberries. Rutin is one of the primary flavonols found in clingstone peaches.

3.3. Medicinal importance of Rutin

In humans, rutin acts as antioxidant, attaches to Fe$^{2+}$, preventing it from binding to hydrogen peroxide, which would otherwise create a highly reactive free radical that may damage cells. Furthermore rutin has been shown to inhibit *in vitro* the vascular endothelial growth factor in subtoxic concentrations, so acts as inhibitor of
angiogenesis\textsuperscript{9}. Although there is a body of evidence for the effects of rutin and quercetin in mice\textsuperscript{10}, rats\textsuperscript{11}, hamsters\textsuperscript{12} and rabbits\textsuperscript{13} as well as in vitro studies\textsuperscript{14}, no clinical studies directly demonstrating significant positive effects of rutin as dietary supplement in humans exist.

Rutin inhibits platelet aggregation, as well as decreasing capillary permeability, making the blood thinner and improving circulation\textsuperscript{15}. Rutin shows anti-inflammatory activity in some animal and in vitro models\textsuperscript{16,17}. Rutin inhibits aldose reductase activity. Aldose reductase is an enzyme normally present in the eye and elsewhere in the body. It helps change glucose into the sugar alcohol sorbitol. Rutin also strengthens the capillaries and therefore, can reduce the symptoms of hemophilia. It also may help to prevent a common, unpleasant-looking, venous edema of the legs\textsuperscript{18}.

Rutin, as ferulic acid, can reduce the cytotoxicity of oxidized LDL cholesterol and lower the risk of heart disease. There is also some evidence that rutin can be used to treat hemorrhoids, varicosis and microangiopathy. Hydroxyethylrutosides, a series of synthetic derivative of rutin, are used in the treatment of chronic venous insufficiency. Rutin has a veterinary use in the management of chylothorax in dogs and cats\textsuperscript{19}.

Among seven flavonoids—quercetin, rutin, morin, acacetin, hispidulin, hesperidin, and naringin, it was observed that the superoxide anions scavenging activity of rutin is the strongest. It also helps change glucose into a sugar alcohol called sorbitol. Thus, rutin is a very important phytochemical. We have investigated twelve edible herbs in search of rich source of rutin. In this chapter, our work on quantitative estimation of rutin in a number of edible plants of North East India and findings on sources of rutin will be described.
3.4. Isolation of Rutin

Solubility of rutin is more in polar solvents. It is more soluble in hot alcohols, less so in hot acetone, and insoluble in hydrocarbons, chlorinated hydrocarbons and ethers. Traditionally Rutin is extracted from the plant sources using aqueous alcohols (typically 70-85% isopropanol), followed by the removal of fats, concentration of the extract, and crystallization of the product. Where the solutions of rutin come into contact with equipment made from iron, calcium or aluminium, further steps may be necessary to recover additional rutin from water-soluble complexes with these elements. Ion exchange resins have also been used for isolation of small quantities of rutin from plant extracts\textsuperscript{20}. Other methods have utilized for extraction of rutin are hot water extraction followed by crystallization from water or ethanol\textsuperscript{21, 22} and use of liquid separators to improve the overall yield\textsuperscript{23}. In this study, rutin was first isolated from of ripe orange. The polar fractions from column chromatography of dried aqueous propanol extract afforded to give pure rutin. The isolated rutin was characterized by \textsuperscript{1}H, \textsuperscript{13}C NMR (COSY, HSQC), ESIMS and IR spectroscopy. The authenticated rutin was used as standard for estimation of rutin in edible herbs.

Structure of Rutin:

The isolated compound was analyzed for C\textsubscript{27}H\textsubscript{30}O\textsubscript{16} by elemental analysis and electro-spray mass spectral (ESIMS) analysis. In the ESIMS, the ion at m/z 649 was assigned to the pseudomolecular ion [C\textsubscript{30}H\textsubscript{50}O+K]\textsuperscript{+}. The IR spectrum of compound AN-3 indicated the presence of hydroxyl group (3423 cm\textsuperscript{-1}), conjugated C=O (1655 cm\textsuperscript{-1}) and C=C (1630 cm\textsuperscript{-1}) moieties in the molecule. In the 1H NMR spectrum of AN-3, singlet at 12.61 and broad singlets at 10.86, 9.70 and 9.27 each integrated to
one proton were assigned to four phenolic hydroxyl group present in the molecule. The \(^1\)H NMR spectrum of AN-3 contained signals at 7.54, 7.55, 6.83, 6.39 and 6.20 and are indicative of aromatic protons. Since the compound gives positive tests for flavonoids\(^2\) and the \(^1\)H NMR spectrum of the compound AN-3 revealed the presence of sugars in it, the compound is probably a flavonoid glycoside. In the \(^1\)H NMR spectrum, the doublet at 5.34 with \(J=7.5\) Hz and integrated to one proton was assigned to H-1 proton of glucose unit present in the molecule. The three proton doublet at 0.99 with \(J=6\)Hz has confirmed beyond doubt that the molecule contains a rhamnose unit. This was further confirmed by an one proton doublet at 5.12 with \(J=2\)Hz. Based on these evidences, the structure of the molecule was assigned as 3-[(4,5-dihydroxy-6-(hydroxymethyl)-3-[(3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy]tetrahydro-2H-pyran-2-yl)oxy]-2-(3,4-dihydroxy phenyl)-5,7-di hydroxy-4H-chromen-4-one or rutin. The structure was further confirmed by its \(^{13}\)C NMR spectrum and 1H-1H Correlating COSY90 spectrum and HSQC spectrum. The COSY90 and HSQC spectra of rutin recorded at 500 MHz revealed the complete coupling network of the protons and carbons in rutin.
The edible herbs taken for study are listed below with code numbers EH-1 to EH-12:

(Assamese names inside bracket)

EH-1: *Centella asiatica* (L.) Urban (Bormanimuni)

EH -2: *Crysanthemium coronarium* L. (Babori)

EH -3: *Paederia scandens* (Lour) Merr (syn. *P. foetida* L.) (Bhedailota)

EH -4: *Portulaca oleracea* L. (Malbhog Khutora)

EH -5: *Amaranthus viridis* Lamk. (Bhat Khutora)

EH -6: *Fagopyrum esculentum* (Dhems)

EH -7: *Hydrocotyle sibtherpiodes* (Xoru Manimuni)

EH -8: *Lucus indica* (Durun)

EH -9: *Stillaria media* (Morolia Xak)

EH -10: *Pouzolozia indica* (Borali Bokua, Dudhmor)
EH -11: *Altermanthera sessilis* (Mati Kanduri)

EH -12: *Oxalis corniculata* L. (Xoru Tengesi)

3.5. A Brief Review of Twelve Edible Herbs Found in Assam and Neighboring Areas

*Centella asiatica* (L.) Urban

Vernacular name of *Centella asiatica* is Bor manimuni (Asm.). A prostrate herb, leaves sub-orbicular, reniform, 1-4 cm in diameter, petioles 1-10 cm long. Inflorescence 3-5 flowered umbels. Flowers sub-sessile, petals obtuse. Mericarps laterally compressed. Common in open up areas, back yard or damp places and eco-fallow rice fields. Flowering occurs in April. Leaves and young shoots are eaten as vegetable. It is a well known mind refresher and considered medicinal in stomach complains. The plant is usually used locally as liver tonic and in making curry. Pounded leaves are used to treat wounds, cuts etc.

*Centella asiatica* from Apiaceae family has been used as traditional herbal medicine in Asiatic countries for hundred of years. This plant is indigenous to warmer regions of both hemispheres, including south east Africa, Asia, Sri Lanka, the pacific islands, Madagascar, Eastern south America, Venezuela, Columbia. It is especially abundant in the swampy areas of India, Iran and Pakistan up to an altitude of 700 m. The major principles are the triterpenes, asiatic acid and medecassic acid and their derived triterpene ester glycoside, asiticoside and madecassoide. Centella asiatica is used in treatment of wounds, various insufficiency of the limbs, certain microbacterial infections and cellulitos.25
Centella asiatica extract is recommended for wound healing and treatment of skin lesions and diseases such as leprosy, lupus, eczema and psoriasis. It is a rasayan brain tonic. It has tranquilizing, sedative and spasmolytic properties.

Antioxidative activity of various extracts from different parts of Centella asiatica including leaves, petiols (stolons) and roots using 3 type of solvents (ethanol, water, and light petroleum) were evaluated using a linoleic acid model system and the thiobarbituric acid test. Results showed that ethanol extract of all parts of Centella asiatica exhibited significantly (P < 0.05) higher antioxidative activity than the water extract, while the light petroleum ether showed negligible activity.

Antimicrobial activity of essential oil of Centella asiatica was studied on six bacteria, viz Escherichia Coli, Bacillus subtilis, B.magaterium, Staphylococcus aureus, Protens vulgaris, Xanthomonas campestris and 6 fungi viz. Aspergilus niger, A.parasticus, Rhizopus oryzae, Candida albicans, Fusarium solani and Colletotrichum musae. The essential oil showed remarkable activity against E. Coli, A. niger, R. oryzae, F. solani, C. albicans and C. musae.

Chrysanthemum coronarium L, family: Asteraceae

Vernacular name of Chrysanthemum coronarium is Babori Sak (Asm.). A small annual erect herb, leaves sub-radical, or alternate above, linearly incised. Inflorescence is a yellow capitulum of nearly 1 cm diameter, with uniseriate and female ray florets and bisexual disc florets. Fruits are angled cypsela. It is commonly cultivated winter season green vegetable. Young leaves and shoots are eaten cooked as vegetable.
The essential oils from aerial parts of *Chrysanthemum coronarium* (Asteraceae) growing wild in two different localities of southern Italy, Lascari (L) Palinuro (P) were determined by hydrodistillation in a 0.13-0.16% yield. The oils were analyzed by GC and GC-MS; 68 constituents amounting to 89.0% of the oil (L) and 43 constituents amounting to 91.6% of the oil (P) were identified. The trans-spiroketal-enol ether 2-(2,4 hexadiinylidene)-1,6-dioxa-spiro[4,4] non-3-ene (trans-tonghaosu) with chrysanthenyl and lyratyl esters and camphor were the main components of the oil.

Amount of vitamin K (P K and M K 4 to 7) contained in edible plants was measured by HPLC-ECR-FL method. As a result of measuring vitamin K content in 41 different types of foods, high concentration (797.92 µg/100g and 509.35 µg/100g) were found in toasted and black tea. Concentration larger than 100µg/100g were noted in garland Chrysanthemum and few other plants.

Volatile compounds of *Chrysanthemum coronarium* L. (Garland) from Korea were isolated and analyzed by simultaneous distillation-extraction and gas chromatography and mass spectroscopy respectively. Myrcene (31.9%) was the most abundant compound, followed by α-bisabolol (16.5%), (E,E-α-farnesene(11.0%)) and (E)-β-farnesene (8.4%). Eighteen aroma-active compounds were detected. Conducted on two GC columns with different polarities.

Nutrient composition of few Chinese vegetables including *Chrysanthemum coronarium* were analyzed. The levels of water, protein, fat, sugars glucose, fructose, sucrose, starch, dietary fiber, organic acids, Na, K, Ca, Fe, Mg, Zn, Vitamin C,
thiamin, riboflavin, niacin, carotenes and energy content are reported for 15 Chinese vegetables including *Chrysanthemum coronarium*.

The antifungal activity of *Chrysanthemum coronarium* was evaluated against 12 agricultural pathogens. Flowerhead oil was active both in contact and head-space in-vitro assays producing hyphal growth inhibition, although there was less activity on faster growing fungi. The main compounds identified in the oil were camphor (29.2%), α-pinene (14.8%), β-pinene (9.5%) and lyratyl acetate (9.8%). The blue colour of the oil was due to the presence of chamazulene (0.5%).

**Paederia scandens** (Lour) Merr (syn, *P. foetida* L.) family:Rubiaceae

Vernacular name of *Paederia scandens* is Bhedailota (Asm.); Bonki repuk (Mishing); Pakhi bendang (Bodo). A slender vine. Leaves opposite, elliptic-ovate. Flowers greyish-purple. Fruits ellipsoids, reddish. The climber is common throughout the state easily available near river banks and bamboo grooves. It has got unpleasant smell when any part is smeared. Flowering occurs during July to October. Leaves, tender twigs are used as green vegetable. A pancake is prepared by pounding it with rice, which is blackish in colour. It is also considered medicinal for stomach ache, gastric and related problems.

The chemical composition of volatile oil from *Paederia scandens* (Lour) Merr was analyzed. 31 components which constitute 77.16% of the volatile oil were identified and quantities were determined. The content of 11 components was higher than 2%. They are: 1-ethonyl pentane, isopenyl acetate, benzaldehyde, Ethyl hexanoate, phenyl methyl formate, phenyl methyl acetate, 2-phenyl ethyl acetate,
5,6,7,7α-tetrahydro-4,4,7γ trimethyl-2(4H)-benzofuranone, penta decanoic acid ethyl ester, hexadecanoic acid, isopentyl decanoate

The essential oil of fresh *Paederia scandens* (Lour) Merr was extracted by general steam distillation, and then separated by GC and their structures were determined by MS. A total of 27 components were identified. The identified constituents represented 99.98% of the peak area of the components of the essential oil of fresh *Paederia scandens* (Lour) Merr. The principal chemical constituents of essential oil of fresh *Paederia scandens* (Lour) Merr were acetic acid (31.14%), 2-methyl-2-buten-1-ol (3.32%), furfural (7.49%), 3-furanmenthol (6.10%), 3-(methyl thio) propionaldehyde (0.6%), linalool oxide (8.45%), trans linalool oxide (10.37%), linalool (3.93), isophorone (1.90%), 5-methyl-6,7-dihydro-5(H)-cyclopentapyrazine (0.72%), epoxylinalol (1%), isoborneol (6.35%), β-fenchyl alc.(7.30%) etc.

*Paederia scandens* have been used in traditional Chinese and Indian medicines. A synergistic Ayurvedic composition for the treatment of rheumatoid arthritis was patented by Kashinath, Joshi Yeshwant. *Paderia scandens* preparation in injectable dosage form for treating rheumatism and its preparation method was patented by Wee, Ketong (China). A Chinese patent was made on Chinese medicinal compositions containing extracts from *Paederia scandens* for treating gout.

*Portulaca oleracea* L. family: Portulacaceae

Vernacular name of *Portulaca oleracea* is Malbhog Khutura; Malbhog sak; Hah thengia (Asm.) A prostrate herb with fleshy glabrous leaves. Branchlets reddish or purple coloured. Flowers small, yellow. Generally occurs in damp and open areas.
Tender shoots and leaves are used as vegetable often mixed with other vegetables. Also used as medicine in liver problem and in Jaundice.

The volatile oil of *Portulaca oleracea* was analyzed by GC-MS. Nineteen compounds were separated among which fifteen compounds were identified\(^\text{39}\). The main composition of the oil are linalool (18.96%) and 2-hexadecen-1-ol,3,7,11,15 tetramethyl (13.55%).

The phytochemical analysis of fresh aerial parts of *Portulaca oleracea* (Portulacaceae), growing in Jordan, using conventional chromatographic procedures resulted in the isolation of \(\beta\)-sitosterol, \(\beta\)-sitosterol glucoside,\(N,N'/\)dicyclohexylurea and allantoin. The last three compounds were isolated for the first time from this plant. The structure elucidation of these compounds was obtained by the use of spectral data (UV, IR, MS, \(^1\)H, \(^{13}\)C- and 2D NMR), X-ray crystallography and by comparison with authentic samples\(^\text{40}\).

An extraction method of effective fraction of *Portulaca oleracea* and industrial uses of effective fraction was discussed in an invention by Chen, Shengsan (China)\(^\text{41}\). The effective fraction contains various components with nutritional, health promoting and medical effects, for example fatty acid (mainly unsaturated fatty acid such as \(\omega\)-3 fatty acid and SL3 fatty acid), noradrenaline, dihydroxy-phenyl ethyl amine, Potassium element, Vitamin E and Vitamin C, \(\beta\)-carotene, glutathione and bioflavonoids. Different solvents are adopted to extract and separate fat-soluble and water-soluble components in the effective fraction, because a part of the components is hydrophilic and the other part is lipophilic. The components can be used for medicine, health care, food, beverage and cosmetic.
The repeated column chromatographic separation of EtOH extract of *Portulaca oleracea* afforded seven compounds. The structure of these isolates were identified as bergapten, umbelliferone, daidzein, genistein, protocatechuic acid, ferulic acid, gallic acid by the analysis of physico-chemical properties and spectral data. Their antioxidant properties were evaluated in the DPPH assay\(^4\).

**Hydrocotyle sibthorpioides Lam. family: Apiaceae**

Vernacular name of *Hydrocotyle sibthorpioides* is Haru manimuni (Asm.). A diffuse prostrate herb. Leaves small, hispid, orbicular, cordate, sub-entire or lobed; petiole 2-10 cm long. Inflorescence 8-10 flowered umbels; peduncles very short. Bracts minute; flowers sub-sessile. Fruits orbicular. Found generally in open areas or damp places. Flowering occurs during April-May. Young leaves and shoots are cooked as vegetable specially with small fishes, also in preparing chutney. Leaves are used as liver tonic and in healing wounds of man and animals.

The essential oils of two species of Hydrocotyle (Apiaceae), *Hydrocotyle javanica* Themb and *Hydrocotyle sibthorpioides* Lam. were analysed by GLC. Monoterpens, sequiterpenes and phenols were detected in these herbs\(^4\). Seven new oleanane-type triterpenoid saponins, hydrocotyloside and one known saponin, Udosaponin B were isolated from methanol extract of the whole plant of *Hydrocotyle sibthorpioides*\(^4\).

A method for determination of total flavones in *Hydrocotyle sibthorpioides* Lam. and *Hydrocotyle sibthorpioides* Lam. var.batrachium(Hance) Hand –Mazz ex Shan was established and contents of total flavones in two plants were determined and compared. The total flavones were extracted with 70% EtOH for 1 h under reflux at
85-90 °C and determined by UV spectrophotometry, with rutin as standard. The detection wavelength was 510nm. The content of total flavones was 4.1077 mg/g in *Hydrocotyle sibthorpioides* Lam., and 14.0273 mg/g in *Hydrocotyle sibthorpioides* Lam. Var. batrachium (Hance) Hand-Mazz ex Shan. The content of total flavones was greatly different in two plants.  

Oleanane-type triterpenoidal saponins, hydrocosisaponins A-F (1-6) along with a known saponin, hydrocotyloside VII (7) were isolated from *Hydrocotyle sibthorpioides*. Their structures were established on the basis of spectroscopic analyses including NMR spectroscopic techniques (13C, 1H, COSY, HMBC, TOCSY and NOESY).

*Stellaria media* (L) Villaris, family: Caryophyllaceae

Vernacular name of *Stellaria media* is Morolia (Asm.). A diffused herb with striate branchlets. Leaves decussate, lower leaves long petioled and upper ones subsessile. Flowers small, pale white, in dichasial cymes. A common weed of winter season crops. Tender twigs are eaten as vegetable, and are also used as medicine in stomach disorders.

Aerial parts of *Stellaria media* contained carboxylic acids, coumarins, hydroxycoumarins, glycosides and saponins. Use of the plant in folk medicines may be dependent on the presence of these compounds.

Protein is isolated from fresh leaves of alfalfa and *Stellaria media* by the Mucciarelli and Yan Wanhua method with modification. The composition of the protein prepared by this process was analyzed. The result showed that protein content was over 68%. Proper amount of coarse fiber (2-3g/100g D.W), Coarse fat (0.5-
0.9g/100g D.W) and amino acid were found in the protein preparation, the effective compounds. Of the isolated protein meet the demand of edible protein and food additives. The chemical constituents from *Stellaria media* were studied. Column chromatography (Silica gel, C18, and Sephadex LH-20) was used to separate the chemical constituents whose structures were determined by spectral analysis (¹H NMR, ¹³CNMR, MS and IR). The isolated and identified compounds were Cyclo (Leulle), Cyclo(Val-Tyr), α-ethyl-D-pyran-galactoside, uracil, thiamin, ananine, serine, leucine, glycine, threonine, lysine, histidine, praline and γ-aminobutyric acid. All the compounds were isolated from this plant for the first time.

Seven compounds were identified from *Stellaria media*. The compounds were emodin, physcion, questin, 1-hexacosanol, β-sitosterol, daucosterol and kaempferol-3,7-β-L-dirhamnoside. Emodin, physcion questin and kaempferol-3,7-β-L-dirhamnoside were isolated from stellaria genus for the first time.

Total free amino acid in *Stellaria media* was found to be 3.1454%. Human necessary amino acid accounted for 25.7% of the total free amino acids. Glutamic acid accounted for 25% of the total free amino acids.

*Alternanthera sessilis* (L.) R. Br. Ex DC, family: Amaranthaceae

Vernacular name of *Alternanthera sessilis* is Matikaduri; Menmeni; Sakraj (Asm.). A prostrate herb. Leave dark green, elliptic-rhomboid or oblanceolate. Flowers pale white. Bracteoles without spine. A common herb in plains specially in moist localities, crop fields and on road sides. Tender shoots and leaves are used as
vegetable, suitable with fishes, considered highly palatable vegetable. The plant is medicinal in liver troubles.

The medicinal plant *Alternanthera sessilis* contained stigmasterol, β-sitosterol, a saturated aliphatic Hydrocarbon, saturated aliphatic Ester and saturated ester. The hexane extract of the whole herb of *Alternanthera sessilis* R.Br yielded 24-methylenecycloartanol, cycloeucalenol, stigmasterol, β-sitosterol, campesterol, α-spinasterol, 5-α stigmasta-7-enol, and palmitates of preceding steroids. Nonacosane, 16-hentriacontanone, β-sitosterol, stigmasterol and handianol were isolated from *A. Sessilis*. *A. sessilis* is used in traditional Chinese medicine for treating alopecia and dull complexion.

*A. sessilis* is found to be antioxidant that are stable at high temperature and can serve as substitute for synthetic antioxidants.

**Oxalis Corniculata L.**

Vernacular name of *Oxalis Corniculata* is Soru tengeshi (Asm.). A prostrate herb, branchlets creeping, rooting at nodes. Leaves digitately trifoliate. Generally found in damp and open shady places as weed. Flowering occurs in Rainy season. Young shoots & leaves are used as vegetable. It is mildly acidic. The plant is also highly considered as medicinal in dysentery and blood pressure. It is consumed as green vegetable.

From oxalis corniculata, 5,7,4′- trihydroxy-6-C-β-D-glucopyranoside (vitexin), 5,7,4′-trihydroxy-6- C-β-D-glucopyranoside (isovitexin) and vitexin-2′/O-β-glucopyranoside were isolated.
*Oxalis corniculata* is used in hyperglycemia and also as anti hypertensive agents. The anti hypergycemia and antihypertensive agents are suitable for use in food products⁵⁸. *Oxalis corniculata* is given in chinese medicinal preparation ‘Gukang capsule’ for treating fracture, osteoarthritis and asteoporosis. The patent is related to new dosage based on the known Chinese medicinal preparation ‘Gukang capsule’⁵⁹.

The extraction method for total flavonoids from *Oxalis corniculata* were compared and the content of total flavonoid was detected by UV-visible spectrophotometry⁶⁰. *Oxalis corniculata* was extracted by 70% ethanol, 50% ethanol, 70% methanol and 50% methanol for 8, 4, 2 & 2h at 90°C respectively. The content of total flavonoids extracted by 70% ethanol, 50% ethanol, 70% MeOH and 50% MeOH was 17,258 µg/g, 19,879 µg/g, 22,258µg/g and 12863 µg/g respectively. The extraction by 50% ethanol was better than 70% ethanol and extraction by 70% MeOH was better than 50% MeOH.

*Amaranthus viridis* L, family Amaranthaceae

Vernacular name of *Amaranthus viridis* is Khutra Sak; Khuduna (Asm.). An annual erect or semi-erect tender herb. Leaves alternate, exstipulate. Flowers minute, pale white to greenish, in panciles. Fruit is utricle. Mostly prefer wet places. Flowering generally through out the year. Tender shoots & leaves are eaten cooked and considered medicinal, mostly prescribed in urinary problems.

The vitamin, carbohydrate, protein and Ca and Fe contents of various plants, used as vegetables (including *Amaranthes viridis*), were determined⁶¹.

A number of commonly consumed plant foods (including *Amaranthues virdis*) which include green leafy vegetables, roots and tubers, other vegetables and fruits
were analyzed for their total carotenoids spectrophotometrically and separation of provitamin-A carotenoid on HPLC\textsuperscript{62}. \(\beta\)-carotene is the predominant carotenoid in all foods. Green leafy vegetables were found to be the most source of provitamin A.

Nutritional (ascorbic acid, dehydroascorbic acid and carotenes), antinutritional and toxic components (Oxalic acid, nitrate and erucic acid) were found in sixteen popular species of wild edible plants which are collected for human consumption in South East Spain\textsuperscript{63}. Ascorbic acid and dehydroascorbic acid content were very high in several species, corotenoid content of \textit{Amaranthus viridis} was found to be 15.4 mg/100g. Nitrate contents of \textit{Amaranthus viridis} L was found to be 597 mg/100g.

From \textit{Amaranthus viridis} L., three flavanoid compounds were isolated and identified. A triterpene saponin glycoside was also isolated and identified. The saponin content was found to be 0.4%. It possessed variable degrees of antiinflammatory, antipyretic and hepatoprotective effects and only the aqueous extract showed an antihelminthic effect\textsuperscript{64}.

\textit{Fagopyrum esculentum} (Buckwheat) Moench, family Polygonaceae

Vernacular name of \textit{Fagopyrum esculentum} is Chutia Lofa; Dhemsi Sak (Asm.). A glabrous annual erect herb, attaining about 90 cm height. Leaves triangular, cordate. Flowers white. The plant is cultivated for foliage and seeds, but in most cases runs into wild. Young leaves and shoots are eaten cooked as vegetable. Seeds are also eaten. Buckwheat flour is mixed with water and adjust pH to other than 4-5 to obtain the precipitate for manufacturing feed. The feed is useful for lowering the blood cholesterol\textsuperscript{65}.
Trace elements and starch content of Buckwheat (Fagopyrum esculentum) were determined and the nutritional value of Buckwheat food (such as protein quality, polyphenols, trace elements) especially in disease was discussed\textsuperscript{66}. The content of Cu, Zn, Mn, Mo and Se was determined in Italian buckwheat samples. Following antoclaving similar and traditional shilling process the content of fast digestible starch decreased and that of resistant starch increased with increasing no of cycles. Total amylase content in buckwheat product was 24-35%.

An analytical method for flavonoids present in the seed extract of buckwheat (\textit{Fagopyrum esculentum} Moench) using HPLC and a photodiode array detector and interfaced to an electrospray ionization mass spectrometer has been developed.

Structural information about the flavonals was obtained from retention time characteristics, the UV-visible spectra and the mass spectra without the need to isolate the individual compounds\textsuperscript{67}. The methanol extract of buckwheat contained principally four flavonol glycosides: rutin, guercetin, Kaempferol-3-rutinoside and a trace quantity of a flavonol triglycoside.

Nutrient ingredient of buckwheat from Xigaze district of Tibet were determined and analyzed. The result of data indicates that buckwheat has a lot of nutritional compounds, such as protein, fats, vitamin, amino acid and mineral elements. Comparing with other grain crops (Wheat, rice etc) buckwheat has higher content in protein and fat and is rich in \(V_{B1}, V_{B2}\). Many mineral elements of buckwheat are easily absorbed. Buck wheat has 8 essential amino acids with reasonable proportion\textsuperscript{68}. 

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The interest of polyphenolics as therapeutic agents against diseases involving radical damage is growing. The phenolic contents of the hulls and flour from the seeds of *Fagopyrum esculentum* (French variety ‘La Harpe’) (total phenols, flavonoids, total flavanols, oligomeric pranthocyanidins) are compared with the antioxidative effects against reactive oxygen species: Hydrogen peroxide, hypochlorous acid, superoxide anion. The higher efficiency of the flour extract can be related to its higher flavanolic content rather than to flavonoids which are predominant in the hull extraction\textsuperscript{69}.

*Leucus indica* (L) [*Llinifolia* (Roth) Spreng *L lavandulifolia* Sn], family- *Lamiaceae*

5,7,4'-trihydroxy-3'-methoxy flavone; 5,7,4'-tri hydroxy flavone, 5,7,3',4'-tetrahydroxy flavone, β-sitosterol 3-O-D-glcoside and a mixture of β-sitosterol and stigma sterol were isolated from the methanol extract of *Leucus indica* (L)\textsuperscript{70}.

The anti inflammatory activity of the methanol extract of *Leucus lavandulifolia* was evaluated on different experimental models of inflammation in rats. The extract has been found to possess significant, inhibitory activity against carrageenin, histamine, serotonin, and dextran induced hind paw edema in rats. The effect produced by extract was comparable to that of phenyl butazone and a prototype, non-steriodal anti inflammatory agent\textsuperscript{71}.

Two flavones, acacetin and chrysoeriol were isolated from chloroform extract of the aerial parts of *Leucus lavandulifolia*\textsuperscript{72}.

Aqueous leaf extract of *Leucus lavandulifolia* and achyranthus aspera at higher concentration inhibited the seed germination and seedling growth of *Pennisetum amaricanum*. The investigation revealed that inhibitory substances present in the leaf
extracts of these weed caused much stronger inhibition on seed germination, leaf sheath elongation and root growth\textsuperscript{73}.

A flavonoid glycoside from aerial parts of Leucus lavandulifolia, chrysoeriol-6\textsuperscript{H}-(O'AC)-4\textsuperscript{H}-\beta-glucoside has been isolated\textsuperscript{74}.

\textit{Pouzolzia zeylanica} (L) Benn & Brown [p.indica (L) Gaertn], family –Urticaceae

Vernacular name of \textit{Pouzolzia zeylanica} is Borali Bokua, Dudhmor (Asm.). An erect or semi-erect herb, attaining about 40 cm height. Leaves ovate to lanceolate, acute to acuminate. Flowers minute, in axillary clusters. The plant is a common upland weed of shaded and marshy situations. Young leaves and shoots are eaten cooked as vegetable.

The chloroform extract of \textit{Pouzolzia indica} afforded a prenylated isoflvene, 5-methoxy-4 hydroxy-2\textsuperscript{H}, 2\textsuperscript{H}-dimethyl pyrano (3\textsuperscript{H},4\textsuperscript{H},7,8) isoflavone studies. The compound exhibited potent antimicrobial and antifungal activities\textsuperscript{75}.

Collagen formation accelerator active oxygen & cavenger and hyaluronidase inhibitor are obtained by extraction of \textit{P. Zeylanice} with water and /or hydrophilic organic solvent. Thus a cosmetic emulsion containing the extract showed high efficacy in treatment of rough skin of women\textsuperscript{76}. 
3.6. Photographs of 12 edible herbs examined

*Centella asiatica*  
*Crysanthemum coronerium*

*Paederia scandens*  
*Portulaca oleracea*

*Amaranthus viridis*  
*Fagopyrum esculentum*
Hydrocotyle sibthorpioides

Leucas indica

Stellaria media

Pouzolzia india

Alternanthera sessilis

Oxalis corniculata
3.7. Detection and Estimation of Rutin

Although there are several colour tests are known for detection of rutin, its reactions are generally those of flavonoids and not specific for rutin. It forms coloured complexes with the salt of many heavy metals and this property is used in some analytical procedures. Chromatography is a valuable technique for detection of rutin, and paper chromatographic methods have been utilized both for detection of rutin in quantities as small as 10 µg, and for its spectrophotometric estimation following elution of spots from chromatograms\textsuperscript{77}. A gravimetric procedure and spectrophotometric analysis of rutin-aluminium chloride complex have also been described\textsuperscript{78}. Thin-layer chromatography on silica gel has been used for the identification and estimation of rutin and quercetin by UV spectrophotometric analysis after elution from the chromatogram, rutin being measured at 273 nm and quercetin 317 nm. Careful preparation of calibration graphs is necessary since only around 50\% of the flavonoids are recovered from the adsorbent\textsuperscript{79}. Similar methods in which the eluted rutin was reacted with p-aminobenzoic acid and NaNO\textsubscript{2} and the coloured solution measured at 420 nm\textsuperscript{80} or the eluted rutin measured at 363 nm\textsuperscript{81} have also been described. Inevitably, with the increased use of HPLC, particularly over the last a few decades, HPLC separations and determinations of rutin have been developed, utilizing reversed phase systems with either C-18\textsuperscript{82-85} or C-8\textsuperscript{86} columns and moving phases of combinations of acetic acid, water, methanol, and acetonitrile. The moving phase may be buffered with potassium dihydrogen orthophosphate\textsuperscript{87} or with citrate\textsuperscript{88} or THF may be used as an organic modifier in place of methanol\textsuperscript{26}. Detection is by UV absorption
in the range 312-390 nm, depending on whether the system is used specifically for rutin or for flavonoids in general.

Capillary Electrophoresis (CE) is a high-resolution technique, which enables the quick and accurate determination of rutin from complex samples. High performance thin layer chromatography (HPTLC) is an enhanced form of TLC to increase the resolution and to allow more accurate quantitative measurements. Normal and reversed phase HPTLC techniques have been used frequently for accurate determination of rutin and related flavonoids now a day.

In this study rutin is estimated in the selected edible herbs by HPLC. Luna C18(2) column was used and solvent system used were methanol/water 1:1 (0-10 min) and 7:3 (10-20 min); flow-rate of 1.0 ml/min. Results of HPLC studies are summarized in Table 3.4. The herbs were also investigated for essential elements (iron, calcium and zinc) and harmful elements (cadmium and lead). These results are shown in Table 3.5, 3.6, 3.7 and Fig. 3.5, 3.6, 3.7. The herbs were further evaluated against Alternaria tenuissima and this result is shown in Table 3.8.

3.8. Experimental

3.8.1. General Experimental procedures

$^1$H and $^{13}$C NMR spectra were recorded on AVANCE DPX500 NMR Spectrometer at 500 MHz and 125 MHz, respectively. IR spectra were recorded on a Perkin–Elmer FT IR spectrophotometer. Micro-analyses were obtained on a Perkin Elmer 2400 elemental analyzer. EIMS was recorded on Bruker Esquire3000 mass spectrometer. Prior to use all solvents were distilled. HPLC chromatograms were recorded in Agilent HPLC System using Luna C18(2) columns.

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3.8.2. Isolation of Rutin

About 5 kg of ripe commercial orange were crushed and juice obtained was then heated gently at 50°C for 6 hours. Then the debris were filtered from the juice. The clear juice was then washed thoroughly with hexane, chloroform and ethyl acetate. The washed juice was lyophilized till all water were removed. The yield of the crude extract was 15 g.

Slurry preparation:

Dried flower part was dissolved in distilled water few ml of methanol is added. Then required amount of silica gel (60-120 mesh) was added. Methanol was removed from the mixture using rotavapour and keeping it in lyoplilizer for 6 hr.

Purification by column:

The fractions are collected by using the following solvent system: Chloroform, ethanol- chloroform (10%, 20%, 30%, 40%, 50%, 75%) and finally ethanol and total 35 fractions are collected.

On examination of the fractions it was found that the fractions 15 to 38 had only one compound and these fractions were combined. On evaporation of the combined fraction yielded 230 mg of light yellow pure crystalline rutin. IR, ESIMS, $^1$H-$^1$H Correlating COSY90 spectrum and HSQC spectrum. The COSY90 and HSQC spectra were recorded for it.
Spectral data of rutin or 3-((4,5-dihydroxy-6-(hydroxymethyl)-3-[(3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy]tetrahydro-2H-pyran-2-yl)oxy)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one

mp 242° C; [α]D = +35° (ethanol, Temp: 30° C, wavelength: 589.3); IR (KBr, cm⁻¹): 3423, 2928, 1655, 1630, 1572, 1505, 1456, 1361, 1295, 1203. 11767, 1090, 1065, 1040, 1014, 938, 875, 833, 807, 723, 657, 597.; ¹H NMR (DMSO-d6, assigned by COSY90, Chemical shift δ in ppm): 12.61(Phenolic OH, C-5), 10.86 (Phenolic OH, C-7), 9.70 (Phenolic OH, C-4′), 9.27 (Phenolic OH, C-3′), 7.54(d, J=9, H-6′), 7.55(H-1′), 6.83 (d, J=9, H-5′), 6.39(1H, dd, J=3, H-8), 6.20 (1H, J=1, H-6), 5.34(1H, d, J=7.5, H-1G), 5.12(1H, d, J=2, H-1, R), 4.37(H-3R), 3.72-3.09(H-3G, H-6G, H-5G, H-5R, H-2R, H-4G, H-4R, H-2G), 2.51(H-2G), 1.00(3H, d, J=6, H-6R). G indicates glucopyranosyl unit, R indicates Rhamnopyranosyl unit; ¹³C NMR (DMSO-d6, assigned by HSQC, Chemical shift δ in ppm): 177.81(C₄), 164.51(C₇), 161.66(C₆), 157.06(C₂), 156.86(C₃), 148.86(C₄), 145.19(C₃), 133.73(C₃), 126.06 (C-6′), 121.61(C₁), 116.70(C₂), 115.66(C₃), 104.40(C₁₀), 101.19(C₁R), 98.70(C₆), 97.28(C₁G), 93.55(C₈), 76.43(C₃G), 75.69(C₃G), 74.01(C₂G), 73.69(C₄R), 72.14(C₃R), 71.96(C₂R), 70.85(C₄G), 63.70(C₅R), 62.31(C₆G), 18.19(C₆R). G indicates glucose unit, R indicates Rhamnose unit; ESIMS m/z at 649 [C₂₇H₃₀O₁₆+K]⁺, Analysis found C 53.12 % H 4.90 %; C₂₇H₃₀O₁₆ requires C 53.02 % H 4.92 %.
3.8.3. Extraction of plant material (edible herbs):

Collection of plant material

The plants were collected, identified and supplied by Dr. Iswar Barua, AAU, Jorhat. The plant materials were thoroughly washed to remove any contamination and then shed dried under air circulation. Then these were grounded to powder form with a Wiley mill.

General procedure of Extraction of plant material

Extraction of the plant material was done in methanol at room temperature by putting the plant material in the solvent (200 ml) and keeping it for a period of 2 days. Then, the plant material was filtered out and the marc was again immersed in 200 ml methanol. This process was repeated three times. The methanol extract obtained were combined and evaporated to dryness under reduced pressure using a rotary evaporator at 50°C. The gummy mass obtained has been subjected for HPLC analysis to check the presence of rutin and quercetin as per procedure reported in the literature. The samples were filtered through a Millipore HA (0.45 µm) membrane filter prior to injection. In all samples injection volume was 15 µl.

Analysis of flavonoids in the extract:

Analysis of flavonoids were done as per procedure reported in the literature. HPLC analysis was done on an Agilent 1100 series HPLC system that consists of online degasser, quaternary pump, auto sampler, thermostat compartment and a PDA detector. The analysis was done using a stainless steel column with 250 mm X 4.6 mm i.d. and packed with Luna C-18(2) column, 5µ particle size. Detection was performed at a wavelength of 340 nm at room temperature. The mobile phase consisted
methanol/water 1:1 (0-10 min) and 7:3 (10-20 min); flow-rate of 1.0 ml/min. A 15 μL volume of sample was injected for each separation.

The solvent used was of HPLC (gradient) grade. The mobile phase was filtered through 0.45-μm nylon membrane prior to use. The mobile phase flow rate was kept at 1.0 ml/min with 130 bar pressure and monitored at 340 nm using PDA detector.

A stock solution was prepared by dissolving 5 mg of authentic rutin in 5 ml water. A standard calibration curve was drawn using stevioside standard solution of following concentrations: 6μg/15μL, 9μg/15μL, 15μg/15μL, 20μg/15μL, 30μg/15μL. This standard curve was used to determine the rutin contents in the extract made from edible herbs sample. Finally, the percentage of rutin in dry plant material was calculated using this curve.

Estimation of quercetin was done in the same way as described above.

The extracted mass (2 mg) was dissolved in mobile phase and filtered through a Millipore HA (0.45 μm) membrane filter. Flavonoids, i.e. rutin, and quercetin, were identified on the basis of comparison of retention time of these running under the same instrumental condition recorded with authentic samples.
The result of this analysis are shown in following Tables

Table 3.1. Quercetin Standard from HPLC

<table>
<thead>
<tr>
<th>Standard Quercetin</th>
<th>Amount in μg/15 μL</th>
<th>Pick area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>7</td>
<td>71267</td>
</tr>
<tr>
<td>Standard</td>
<td>12</td>
<td>127416</td>
</tr>
<tr>
<td>Standard</td>
<td>15</td>
<td>165039</td>
</tr>
<tr>
<td>Standard</td>
<td>20</td>
<td>214189</td>
</tr>
<tr>
<td>Standard</td>
<td>24</td>
<td>244744</td>
</tr>
</tbody>
</table>

**Fig. 3.1.** Graph for HPLC peak area vs amount of Quercetin
Table 3.2. Amount of Quercetin present in various edible herbs

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Amount of extract injected μg/15μL</th>
<th>HPLC peak Area</th>
<th>Amount of Quercetin present in extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH-1</td>
<td>6</td>
<td>2141 ± 2.58</td>
<td>0.22</td>
</tr>
<tr>
<td>EH-2</td>
<td>6</td>
<td>5413 ± 2.01</td>
<td>0.6</td>
</tr>
<tr>
<td>EH-3</td>
<td>6</td>
<td>3251 ± 2.25</td>
<td>0.35</td>
</tr>
<tr>
<td>EH-4</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EH-5</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EH-6</td>
<td>6</td>
<td>236912 ± 1.25</td>
<td>1.8</td>
</tr>
<tr>
<td>EH-7</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EH-8</td>
<td>6</td>
<td>7113 ± 1.53</td>
<td>2.5</td>
</tr>
<tr>
<td>EH-9</td>
<td>6</td>
<td>1067984 ± 2.69</td>
<td>0.7</td>
</tr>
<tr>
<td>EH-10</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EH-11</td>
<td>6</td>
<td>2425 ± 1.09</td>
<td>0.6</td>
</tr>
<tr>
<td>EH-12</td>
<td>6</td>
<td>41548 ± 1.56</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 3.3. Rutin Standard from HPLC

<table>
<thead>
<tr>
<th>Standard</th>
<th>Amount in μg/15 μL</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Rutin</td>
<td>6</td>
<td>7000152</td>
</tr>
<tr>
<td>Standard Rutin</td>
<td>9</td>
<td>10017241</td>
</tr>
<tr>
<td>Standard Rutin</td>
<td>15</td>
<td>15804771</td>
</tr>
<tr>
<td>Standard Rutin</td>
<td>20</td>
<td>21670023</td>
</tr>
<tr>
<td>Standard Rutin</td>
<td>30</td>
<td>31638480</td>
</tr>
</tbody>
</table>
Table 3.4. Amount of Rutin present in various edible herbs

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Amount of Extract injected μg/15μL</th>
<th>HPLC peak Area</th>
<th>Amount of Rutin present in plant extract</th>
<th>Percentage of Rutin in plant extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH-1</td>
<td>6</td>
<td>23972 ± 1.34</td>
<td>0.21</td>
<td>1.98</td>
</tr>
<tr>
<td>EH-2</td>
<td>6</td>
<td>14153 ± 1.65</td>
<td>0.15</td>
<td>1.00</td>
</tr>
<tr>
<td>EH-3</td>
<td>6</td>
<td>17317 ± 2.01</td>
<td>0.19</td>
<td>0.39</td>
</tr>
<tr>
<td>EH-4</td>
<td>6</td>
<td>16215 ± 1.39</td>
<td>0.17</td>
<td>0.93</td>
</tr>
<tr>
<td>EH-5</td>
<td>6</td>
<td>36765 ± 1.98</td>
<td>0.38</td>
<td>1.84</td>
</tr>
<tr>
<td>EH-6</td>
<td>6</td>
<td>1865440 ± 2.01</td>
<td>1.6</td>
<td>8.76</td>
</tr>
<tr>
<td>EH-7</td>
<td>6</td>
<td>8375 ± 1.56</td>
<td>0.09</td>
<td>0.50</td>
</tr>
<tr>
<td>EH-8</td>
<td>6</td>
<td>13598 ± 1.45</td>
<td>0.14</td>
<td>0.23</td>
</tr>
<tr>
<td>EH-9</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EH-10</td>
<td>6</td>
<td>643341 ± 1.13</td>
<td>0.7</td>
<td>0.33</td>
</tr>
<tr>
<td>EH-11</td>
<td>6</td>
<td>82757 ± 2.45</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>EH-12</td>
<td>6</td>
<td>1683516 ± 2.98</td>
<td>1.4</td>
<td>1.65</td>
</tr>
</tbody>
</table>
3.9. A brief discussion on Fe, Ca and Zn metals

Iron is a chemical element with the symbol Fe (from Latin: ferrum) and atomic number 26. Iron plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin; these two compounds are common oxygen transport proteins in vertebrates. Iron is also the metal used at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals.

Iron is abundant in biology. Iron-proteins are found in all living organisms, ranging from the evolutionarily primitive archaea to humans. The color of blood is due to the hemoglobin, an iron-containing protein. The most commonly known and studied iron compounds used in biology are the heme proteins: examples are hemoglobin, myoglobin, and cytochrome P450. These compounds can transport oxygen, build enzymes, and be used in transferring electrons. The iron-sulfur clusters are pervasive and include nitrogenase, the enzymes responsible for biological nitrogen fixation. Some examples of other iron containing metalloproteins are ferritin and rubredoxin.94-96

Fig. 3.3. Structure of Heme b, in the protein addition ligand(s) would be attached to Fe
**Calcium** is the chemical element with the symbol Ca and atomic number 20. It has an atomic mass of 40.078 amu. Calcium is a soft gray alkaline earth metal, and is the fifth-most-abundant element by mass in the Earth's crust. Calcium is also the fifth-most-abundant dissolved ion in seawater by both molarity and mass, after sodium, chloride, magnesium, and sulfate.

Calcium is essential for living organisms, in particular in cell physiology, where movement of the calcium ion $\text{Ca}^{2+}$ into and out of the cytoplasm functions as a signal for many cellular processes. As a major material used in mineralization of bones and shells, calcium is the most abundant metal by mass in many animals.

Calcium is an important component of a healthy diet and a mineral necessary for life. Calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life. Approximately 99 percent of the body's calcium is stored in the bones and teeth. The rest of the calcium in the body has other important uses, such as some exocytosis, especially neurotransmitter release, and muscle contraction. In the electrical conduction system of the heart, calcium replaces sodium as the mineral that depolarizes the cell, proliferating the action potential. Long-term calcium deficiency can lead to rickets and poor blood clotting and in case of a menopausal woman, it can lead to osteoporosis, in which the bone deteriorates and there is an increased risk of fractures. While a lifelong deficit can affect bone and tooth formation, over-retention can cause hypercalcemia (elevated levels of calcium in the blood), impaired kidney function and decreased absorption of other minerals.\textsuperscript{97-99}

**Zinc** is a metallic chemical element; it has the symbol Zn and atomic number 30. It is the first element in group 12 of the periodic table. Zinc is an essential mineral
of "exceptional biologic and public health importance. Zinc deficiency affects about two billion people in the developing world and is associated with many diseases. In children zinc deficiency causes growth retardation, delayed sexual maturation, infection susceptibility, and diarrhea, contributing to the death of about 800,000 children worldwide per year.

Zinc is an essential trace element, necessary for plants, animals and microorganisms. Zinc is found in nearly 100 specific enzymes, serves as structural ions in transcription factors and is stored and transferred in metallothioneins. It is "typically the second most abundant transition metal in organisms" after iron and it is the only metal which appears in all enzyme classes.

In proteins, Zn ions are often coordinated to the amino acid side chains of aspartic acid, glutamic acid, cysteine and histidine. There are 2–4 grams of zinc distributed throughout the human body. Most zinc is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye. Semen is particularly rich in zinc, which is a key factor in prostate gland function and reproductive organ growth.

In humans, zinc plays "ubiquitous biological roles". It interacts with "a wide range of organic ligands", and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression. It also regulates apoptosis. In the brain, zinc is stored in specific synaptic vesicles by glutamatergic neurons and can "modulate brain excitability". It plays a key role in synaptic plasticity and so in learning. However it has been called "the brain's dark horse" since it also can be a neurotoxin, suggesting
zinc homeostasis plays a critical role in normal functioning of the brain and central nervous system.100-103

Fig. 3.4. (A) Zinc Finger protein, (B) human carbonic anhydrase II with Zinc atom at the center

Lead is a main-group element in the carbon group with the symbol Pb (from Latin: plumbum) and atomic number 82. Lead is a soft, malleable poor metal. It is also counted as one of the heavy metals.

Lead, at certain exposure levels, is a poisonous substance to animals as well as for human beings. It damages the nervous system and causes brain disorders. Excessive lead also causes blood disorders in mammals.

Lead is highly poisonous metal (regardless if inhaled or swallowed), affecting almost every organ and system in the body. The main target for lead toxicity is the nervous system, both in adults and children. Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO$_2$) can cause nephropathy, and colic-like abdominal pains. Exposure to high lead levels can severely damage the brain and kidneys in adults or children and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. Lead also damages nervous
connections (especially in young children) and cause blood and brain disorders. Lead poisoning typically results from ingestion of food or water contaminated with lead; but may also occur after accidental ingestion of contaminated soil, dust, or lead-based paint. This causes ineffective heme synthesis and subsequent microcytic anemia. At lower levels, it acts as a calcium analog, interfering with ion channels during nerve conduction. This is one of the mechanisms by which it interferes with cognition. Acute lead poisoning is treated using disodium calcium edetate: the calcium chelate of the disodium salt of EDTA. This chelating agent has a greater affinity for lead than for calcium and so the lead chelate is formed by exchange. This is then excreted in the urine leaving behind harmless calcium.\textsuperscript{104,105}

**Cadmium** is a chemical element with the symbol Cd and atomic number 48. This soft, bluish-white metal is chemically similar to the two other stable metals in group 12, zinc and mercury. Like zinc, it prefers oxidation state +2 in most of its compounds and like mercury it shows a low melting point compared to transition metals.

Cadmium has no known useful role in higher organisms, but a cadmium-dependent carbonic anhydrase has been found in some marine diatoms. Cadmium is also an environmental hazard. Human exposures to environmental cadmium are primarily the result of fossil fuel combustion, phosphate fertilizers, natural sources, iron and steel production, cement production and related activities, nonferrous metals production, and municipal solid waste incineration.

The highest concentration of cadmium is found to be absorbed in the kidneys of humans. The most dangerous form of occupational exposure to cadmium is
inhalation of fine dust and fumes, or ingestion of highly soluble cadmium compounds. Inhalation of cadmium-containing fumes can result initially in metal fume fever but may progress to chemical pneumonitis, pulmonary edema, and death.\textsuperscript{106,107}

3.10. AAS study of different edible herbs (Sample code: EH-1 to EH-12)

AAS studies for determination of Cd, Pb, Ca, Fe and Zn were done by using AAnalyst-100 (Perekin Elemer) instrument. Cd and Pb were found to be Below Detection Limit (BDL) in all the plants. BDL for Cd, Pb, Ca, Fe and Zn are < 0.03, < 0.2, < 0.06, < 0.1 and < 0.02 mg/L respectively. Different plants samples were dried and grinded. 1g of the dry sample of each plant were taken in porcelain crucibles and placed in muffle furnace at 500°C overnight. 5 mg of ash of each sample were dissolved in 20% HCl and warmed to dissolve the residue. The dissolved solutions (containing 5 mg of ash of each sample) were taken in 50 ml volumetric flask and the made up the volumes up to the mark with deionized water. These sample solutions were given for AAS studies. The total content and % of Fe, Ca and Zn are given in the following tables. The amount of Cd and Pb in all the samples are found to be below detection limit (BDL).

3.11. Estimation of Essential elements:

Table 3.5. Fe content in different plants from ASS studies.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Amount of Dry plant (g)</th>
<th>Amount of ash (g)</th>
<th>Fe content from ASS (mg/L)</th>
<th>Fe content in 5 mg of ash (mg)</th>
<th>Fe content in total ash (mg)</th>
<th>% of Fe content in dry plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH-1</td>
<td>1</td>
<td>0.2011</td>
<td>3.2 ± 0.100</td>
<td>0.1600</td>
<td>6.4352 ± 0.01975</td>
<td>0.64 ± 0.00198</td>
</tr>
<tr>
<td>EH-2</td>
<td>1</td>
<td>0.2235</td>
<td>0.55 ± 0.015</td>
<td>0.0275</td>
<td>1.2292 ± 0.00181</td>
<td>0.12 ± 0.00018</td>
</tr>
</tbody>
</table>

164
<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Amount of Dry plant (g)</th>
<th>Amount of ash (g)</th>
<th>Ca content from ASS (mg/L)</th>
<th>Ca content in 5 mg of ash (mg)</th>
<th>Ca content in total ash (mg)</th>
<th>% of Ca content in dry plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH-1</td>
<td>1</td>
<td>0.2011</td>
<td>2.0 ± 0.03786</td>
<td>0.1600</td>
<td>6.4352 ± 0.01975</td>
<td>0.40 ± 0.00001</td>
</tr>
<tr>
<td>EH-2</td>
<td>1</td>
<td>0.2235</td>
<td>5.40 ± 0.0100</td>
<td>0.0275</td>
<td>1.2292 ± 0.00181</td>
<td>1.21 ± 0.00002</td>
</tr>
<tr>
<td>EH-3</td>
<td>1</td>
<td>0.2482</td>
<td>7.0 ± 0.15695</td>
<td>0.0435</td>
<td>2.1593 ± 0.00040</td>
<td>1.74 ± 0.00002</td>
</tr>
<tr>
<td>EH-4</td>
<td>1</td>
<td>0.2654</td>
<td>3.27 ± 0.02082</td>
<td>0.0270</td>
<td>1.4331 ± 0.00015</td>
<td>0.87 ± 0.00002</td>
</tr>
<tr>
<td>EH-5</td>
<td>1</td>
<td>0.2390</td>
<td>4.17 ± 0.01528</td>
<td>0.1135</td>
<td>5.4253 ± 0.00006</td>
<td>1.00 ± 0.00001</td>
</tr>
<tr>
<td>EH-6</td>
<td>1</td>
<td>0.2775</td>
<td>13.0 ± 0.06658</td>
<td>0.0480</td>
<td>2.6640 ± 0.00021</td>
<td>3.61 ± 0.00001</td>
</tr>
<tr>
<td>EH-7</td>
<td>1</td>
<td>0.3150</td>
<td>6.35 ± 0.00577</td>
<td>0.0550</td>
<td>3.4650 ± 0.00020</td>
<td>2.00 ± 0.00002</td>
</tr>
</tbody>
</table>

Table 3.6. Ca content indifferent plants from AAS studies.
<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Amount of Dry plant(g)</th>
<th>Amount of ash(g)</th>
<th>Zn content from ASS(mg/L)</th>
<th>Zn content in 5 mg of ash (mg)</th>
<th>Zn content in total ash (mg)</th>
<th>% of Zn content in dry plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH-1</td>
<td>1</td>
<td>0.2011</td>
<td>0.05 ± 0.01100</td>
<td>0.0025</td>
<td>0.1005 ± 0.00040</td>
<td>0.01 ± 0.00004</td>
</tr>
<tr>
<td>EH-2</td>
<td>1</td>
<td>0.2235</td>
<td>0.07 ± 0.00153</td>
<td>0.0035</td>
<td>0.1564 ± 0.00040</td>
<td>0.02 ± 0.00004</td>
</tr>
<tr>
<td>EH-3</td>
<td>1</td>
<td>0.2482</td>
<td>0.11 ± 0.01217</td>
<td>0.0050</td>
<td>0.2482 ± 0.00010</td>
<td>0.02 ± 0.00001</td>
</tr>
<tr>
<td>EH-4</td>
<td>1</td>
<td>0.2654</td>
<td>0.07 ± 0.00153</td>
<td>0.0035</td>
<td>0.1857 ± 0.00021</td>
<td>0.02 ± 0.00002</td>
</tr>
<tr>
<td>EH-5</td>
<td>1</td>
<td>0.2390</td>
<td>0.05 ± 0.00265</td>
<td>0.0025</td>
<td>0.1195 ± 0.00072</td>
<td>0.01 ± 0.00007</td>
</tr>
<tr>
<td>EH-6</td>
<td>1</td>
<td>0.2775</td>
<td>0.08 ± 0.00206</td>
<td>0.0040</td>
<td>0.2220 ± 0.00010</td>
<td>0.02 ± 0.00001</td>
</tr>
<tr>
<td>EH-7</td>
<td>1</td>
<td>0.3150</td>
<td>0.10 ± 0.00839</td>
<td>0.0050</td>
<td>0.3150 ± 0.00021</td>
<td>0.03 ± 0.00002</td>
</tr>
<tr>
<td>EH-8</td>
<td>1</td>
<td>0.2797</td>
<td>0.08 ± 0.00153</td>
<td>0.0040</td>
<td>0.2237 ± 0.00010</td>
<td>0.02 ± 0.00001</td>
</tr>
<tr>
<td>EH-9</td>
<td>1</td>
<td>0.2010</td>
<td>0.04 ± 0.00263</td>
<td>0.0020</td>
<td>0.0804 ± 0.00025</td>
<td>0.01 ± 0.00003</td>
</tr>
<tr>
<td>EH-10</td>
<td>1</td>
<td>0.2536</td>
<td>0.37 ± 0.27465</td>
<td>0.0255</td>
<td>1.2933 ± 0.00025</td>
<td>0.13 ± 0.00003</td>
</tr>
<tr>
<td>EH-11</td>
<td>1</td>
<td>0.1981</td>
<td>0.15 ± 0.00200</td>
<td>0.0075</td>
<td>0.2971 ± 0.00006</td>
<td>0.03 ± 0.00001</td>
</tr>
<tr>
<td>EH-12</td>
<td>1</td>
<td>0.2130</td>
<td>0.07 ± 0.00100</td>
<td>0.0035</td>
<td>0.1491 ± 0.00010</td>
<td>0.01 ± 0.00001</td>
</tr>
</tbody>
</table>

Table 3.7. Zn content indifferent plants from AAS studies.
**Fig. 3.5.** Fe content in various edible plants

**Fig. 3.6.** Ca content in various edible plants
3.12. Conclusion

In this study we have investigated 12 edible herbs found in Assam. All these herbs have been found to contain rutin and quercetin in different amounts. Highest amount of rutin was found in *Fagpyrum esculantum* and *Oxalis corniculata*. It may be mentioned that rutin was also reported from *Fagpyrum esculantum* grown in Slovenia, Bulgaria and other countries. 108,109 Highest amount of quercetin was found in *Stellaria media*. We have also studied mineral contents for essential elements zinc, calcium and iron. Highest amount of Zinc was found in EH-10 (0.13%). Highest amount of calcium was found in EH-6 (3.6%) and Highest amount of iron was found in EH-10 (1.22 %). Further we have investigated accumulation of toxic element cadmium and lead in these twelve herbs. It was observed that none of these 12 herbs accumulate these two toxic element.

Further we have studied fungi inhibitory properties of the methanolic extract of these twelve herbs against *Alternaria tenuissima*. Among these, extracts of morolia
*(Stillaria media)* was found to have highest anti-fungal activity. The results of antifungal activity for all 12 plants are shown in Table 3.8)

**Table 3.8.** Fungal inhibitory properties of 12 edible herbs

<table>
<thead>
<tr>
<th>Sample name</th>
<th>(µg/ml)</th>
<th>Fungal growth(mg)</th>
<th>Fungal growth (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH-1</td>
<td>200</td>
<td>0.169 ± 0.0234</td>
<td>67.6±0.968</td>
<td>32.4±1.25</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>0.141 ± 0.0130</td>
<td>56.4±0.567</td>
<td>43.6±1.80</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>0.130 ± 0.033</td>
<td>52.0±0.124</td>
<td>48.0±2.01</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>0.123 ± 0.0120</td>
<td>49.2±0.456</td>
<td>50.8±1.09</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.110 ± 0.0150</td>
<td>44.0±0.567</td>
<td>56.0±0.09</td>
</tr>
<tr>
<td>EH-2</td>
<td>200</td>
<td>0.230 ± 0.0270</td>
<td>92.0±0.830</td>
<td>8.0±1.04</td>
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<tr>
<td></td>
<td>400</td>
<td>0.182 ± 0.0265</td>
<td>72.8±0.850</td>
<td>27.2±1.02</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>0.131 ± 0.0130</td>
<td>52.4±0.520</td>
<td>47.6±1.20</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>0.100 ± 0.099</td>
<td>40.0±0.360</td>
<td>60.0±2.01</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.088 ± 0.006</td>
<td>35.2±0.024</td>
<td>64.8±2.03</td>
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<tr>
<td>EH-3</td>
<td>200</td>
<td>0.150 ± 0.0145</td>
<td>60.0±0.452</td>
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<td>0.141 ± 0.134</td>
<td>56.4±0.578</td>
<td>43.6±1.02</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>0.130 ± 0.026</td>
<td>52.0±0.830</td>
<td>48.0±1.02</td>
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<tr>
<td></td>
<td>800</td>
<td>0.116 ± 0.0236</td>
<td>46.4±0.816</td>
<td>53.6±0.09</td>
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<tr>
<td></td>
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<td>0.097 ± 0.045</td>
<td>35.6±0.167</td>
<td>64.4±2.01</td>
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<tr>
<td>EH-4</td>
<td>200</td>
<td>0.191 ± 0.034</td>
<td>76.4±0.148</td>
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<td>0.180 ± 0.067</td>
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<td>600</td>
<td>0.160 ± 0.057</td>
<td>66.8±0.298</td>
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<tr>
<td></td>
<td>800</td>
<td>0.150 ± 0.0123</td>
<td>63.2±0.498</td>
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<tr>
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<tr>
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<tr>
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<td>800</td>
<td>0.120 ± 0.011</td>
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<td>52.0±0.012</td>
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<tr>
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<td>0.100 ± 0.023</td>
<td>40.0±0.856</td>
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</tr>
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<td>EH-6</td>
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<td>0.154 ± 0.980</td>
<td>61.6±0.360</td>
<td>38.4±1.00</td>
</tr>
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<td>400</td>
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<td>46.8±1.00</td>
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<td>600</td>
<td>0.119 ± 0.056</td>
<td>47.6±0.230</td>
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<td>0.087 ± 0.091</td>
<td>34.8±0.360</td>
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</tr>
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<td>0.076 ± 0.001</td>
<td>30.4±0.456</td>
<td>69.6±1.045</td>
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<td>--------</td>
<td>--------</td>
</tr>
<tr>
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<td>1000</td>
<td>0.069 ± 0.019</td>
<td>27.6±0.285</td>
<td>72.4±0.560</td>
</tr>
<tr>
<td>EH-8</td>
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<td>0.132 ± 0.037</td>
<td>52.8±0.100</td>
<td>47.2±0.034</td>
</tr>
<tr>
<td></td>
<td>400</td>
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<td>54.4±0.012</td>
</tr>
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<td>600</td>
<td>0.094 ± 0.037</td>
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<td>83.2±1.060</td>
</tr>
<tr>
<td>EH-10</td>
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<td>58.0±0.398</td>
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<td>47.2±1.030</td>
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<td>52.8±1.002</td>
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<td>56.8±0.032</td>
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<td>0.093 ± 0.011</td>
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<td>62.8±0.109</td>
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<tr>
<td>EH-11</td>
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<td>45.6±0.278</td>
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<tr>
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<td>0.102 ± 0.090</td>
<td>40.8±0.956</td>
<td>59.2±0.23</td>
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</tr>
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<td>0.078 ± 0.045</td>
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<td>68.8±1.020</td>
</tr>
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<td>1000</td>
<td>0.064 ± 0.001</td>
<td>25.6±0.040</td>
<td>74.4±1.023</td>
</tr>
<tr>
<td>EH-12</td>
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<td>0.155 ± 0.011</td>
<td>62.0±0.440</td>
<td>38.0±0.100</td>
</tr>
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<td></td>
<td>400</td>
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<td>54.4±0.835</td>
<td>45.6±0.198</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>0.120 ± 0.011</td>
<td>48.0±0.126</td>
<td>52.0±2.00</td>
</tr>
<tr>
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<td>0.104 ± 0.20</td>
<td>41.6±0.344</td>
<td>58.4±0.217</td>
</tr>
<tr>
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<td>1000</td>
<td>0.087 ± 0.25</td>
<td>34.8±0.845</td>
<td>65.2±0.982</td>
</tr>
</tbody>
</table>
3.13 Spectra of Rutin:

Fig 3.8 $^1$H NMR spectrum of Rutin
Fig 3.9 $^{13}$C NMR spectrum of Rutin
Fig 3.10 COSY90 spectrum of Rutin
Fig3.11 (-) ESIMS of spectrum of Rutin

Fig3.12 IR of spectrum of Rutin
3.14. References


34. Ma, Y., Mao, Y., Fu, J. X. *Xibei Zhiwu Xuebao* 2000, 20, 145.


42. Choi, B., Hong, S. S., Han, X. H. Natural Product Sciences 2005, 11, 229.


109. Atanassova, M., Bagdassarian, V. *Journal of the University of Chemical Technology and Metallurgy*, **2009**, *44*, 201-203.