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Preliminary phytochemical screening of crude methanolic extract of some ethnomedicinal plants used by *Muthuvan* tribe from *Kulachuvayal* tribal colony, Kanthalloor, Idukki district of Kerala, India

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Abstract

Antibacterial activity and phytochemical screening of crude methanolic extract of selected ethnomedicinal plants from Kerala was examined. The plants studied were *Atlantia monophylla*, *Cymbopogon flexuosus*, *Datura stramonium*, *Melia dubia*, *Ruta graveolens*, *Solanum villosum*, *Triumfetta rhomboidea* and *Vitex negundo* which are used for treating various ailments among *Muthuvan* tribe. The methanol extract of the plants were evaluated against four bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). None of them exhibited antibacterial activity. The methanol extract of plants was subjected to qualitative phytochemical tests. The phytochemical screening of plant extract revealed the presence and absence of alkaloids, flavonoids, phenols, tannins, phyllotannins, steroids, terpenoids, carbohydrates, glycosides, saponins. Since the plant possess bioactive compounds, it is reliable to possess large number of therapeutic value and are being employed for the treatment of different ailments among the tribal community.

Keywords: Ethnomedicinal plants, phytochemical tests, *Muthuvan* tribe, Kanthalloor, soxhlet extraction, bioactive compounds

Introduction

Traditional knowledge includes tacit knowledge and practices of tribal communities and is often connected to customs of crop and animal husbandry, fisheries and human health. India abounds in traditional knowledge with its vast geographic expanse spread across different climatic regions. However, this knowledge tends to get extinct as tribal communities get more and more marginalized. The ethnomedicinal plants used for traditional medicine contain various substances that can be used to treat chronic as well as infectious diseases. The curative properties of the medicinal plants are mainly due to the presence of various chemical substances of different composition which occur as secondary metabolites [14].

Idukki district of Kerala, has wide range of forest area. There is a hamlet of '*Muthuvans*' named as *Kulachuvayal* tribal colony in Kanthalloor. Rural communities, especially *Muthuvan* tribe, depends on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade. *Kulachuvayal* tribal colony is one such area where traditional healing systems are still popular among the local people. But so far, few ethnobotanical surveys have been made in this area to know the plants used by the tribes, '*Muthuvan*' who have been inhabited in *Kulachuvayal* colony. The active principles of many drugs found in plants are secondary metabolites [7]. Therefore basic phytochemical screening is vital. This paper deals with an attempt to gather information on some traditional uses of medicinal plants as well as their antibacterial activity and phytochemical screening.

Materials and Methods

Plant materials collection

Plants for the present study were collected on the basis of their medicinal use. Fresh plants were collected from the *Kulachuvayal* tribal colony in Kanthalloor, Kerala. The ethnobotanical data such as, local name, mode of preparation and medicinal uses were collected through discussions among the tribals in their local language (Tamil).

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Preparation of methanol extract

The whole plants were oven dried at 80 °C and ground into fine powder using mortar and pestle. 5g of each of the powdered plant materials were extracted in a soxhlet extractor containing 50ml of 99% methanol. The resulting extracts were evaporated under reduced pressure^[4].

Antibacterial screening

The methanol extracts of 8 plants were screened against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Preparation of inoculums

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of peptone water for bacteria that were incubated for 2 hours at 37 °C for attaining enough turbidity.

Antibacterial susceptibility test

The disc diffusion method was used to screen the antimicrobial activity using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and inoculum suspension was swabbed uniformly. It was then allowed to dry for 5 minutes. The different concentrations of extracts (15 and 30 µL/disc) were loaded on 6mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes. The plates were then kept for incubation at 37 °C for 24 hours.

Phytochemical screening of extracts

Screening of the above medicinal plants for phytochemical various constituents were carried out using standard methods as described in Table 1.

Table 1: Preliminary phytochemical tests for plant extracts

	Phytoconstituents Test	Observation
Alkaloids	2ml extract + 2% H ₂ SO ₄ + heat few drops of Wagner's reagent	Reddish brown precipitate
Flavonoids (Ammonium Test)	2ml extract + 1ml dilute NH ₄ solution (1%)	Yellow colouration
Phenols (Ellagic Acid Test)	2ml extract + few drops of glacial acetic acid (5%) + few drops of NaNO ₂ (5%)	Neither the solution turn muddy nor did niger brown precipitate
Tannins drops of (Ferric Chloride Test)	1ml extract + distilled water + 2 FeCl ₂	Transient greenish to black colour
Phyllotannins	1ml extract + aqueous HCl (1%) + heat	Red colour
Steroids (Salkowski Test)	2ml extract + 2ml chloroform + 2ml Conc.H ₂ SO ₄	Chloroform layer appears red and greenish yellow fluorescent colour in acid layer
Terpenoids (Salkowski Test)	2ml extract + 2ml chloroform + 3ml Conc. H ₂ SO ₄	Reddish brown colouration
Carbohydrates (Fehling's Test)	Fehling A + Fehling B + few drops of extract + heat	Brick red coloured precipitate
Glycosides (Keller-Killani Test)	2ml extract + glacial acetic acid + 1 drop FeCl ₃ (5%) + Conc. H ₂ SO ₄	Reddish brown colour at the junction Upper layer into bluish Green
Saponins (Foam Test)	2ml extract + 20ml distilled water Shaken for 15 minutes	Layer of foam forms
Proteins (Biuret Test)	1ml extract + 1ml NaOH (10%) + heat + CuSO ₄ (0.7%)	Purple violet colour

Results

Antibacterial activity

All the bacterial strains are found to be resistant against the methanol extract of the ethnomedicinalplants. The results were shown in Fig. 1.

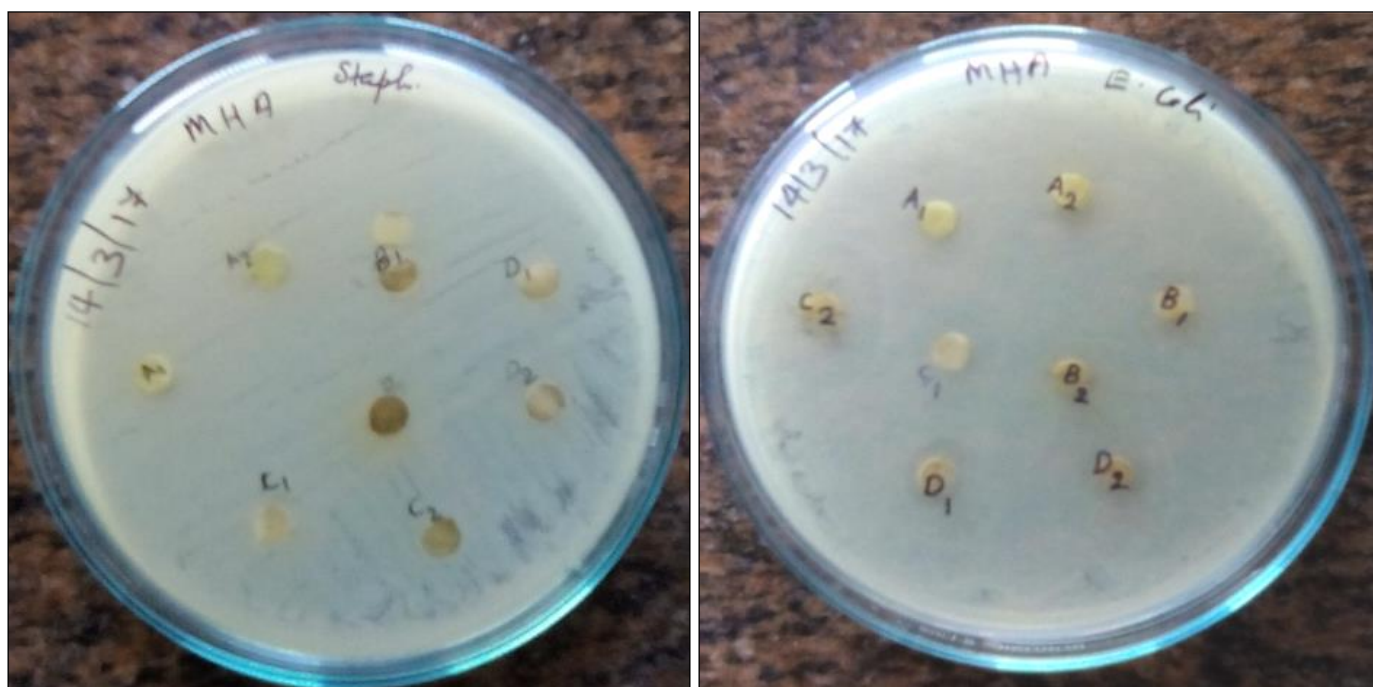




Fig 1: Antibacterial activity of methanol extract of plants against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Phytochemical Screening

Data shown in Table 2 shows screening of methanol extracts of different ethnomedicinal plants. These tests revealed the presence and absence of alkaloids, flavonoids, phenols,

tannins, phyllotannins, steroids, terpenoids, carbohydrates, glycosides, saponins and proteins. These compounds have significant application against human pathogens, including those that cause infections.

Table 2: Phytochemical constituents of eight ethnomedicinal plants studied

Plants	Alkaloids	Flavonoids	Phenols	Tannins	Phyllotannin	Steroids	Terpenoids	Carbohydres	Glycosides	Saponins	Proteins
<i>Atlantia monophylla</i>	-	-	-	+	-	-	+	-	+	-	-
<i>Cymbopogon flexuosus</i>	-	-	+	+	-	-	+	-	-	-	-
<i>Datura stramonium</i>	-	-	+	+	-	-	+	-	+	-	-
<i>Melia dubia</i>	-	-	-	-	-	-	+	+	+	-	-
<i>Ruta graveolens</i>	-	-	+	-	-	+	-	-	+	-	-
<i>Solanum villosum</i>	+	-	+	+	+	-	+	-	-	+	-
<i>Triumfetta rhomboidea</i>	+	+	+	+	-	+	-	-	+	-	-
<i>Vitex negundo</i>	-	-	+	+	-	-	+	-	+	-	-

Discussion

The biological activity of the plant depends on many factors like, plant part, geographical source, soil conditions, and time of the harvest, moisture and post-harvest process methods. For example, high temperature during tissue grinding may denature certain chemical constituents. Different concentrations of solvent or different solvents alone or combinations are used for the maximum recovery of bioactive compounds, because different plants constitute different compositions of active compounds^[35].

The different concentrations (15 μ L and 30 μ L) of the methanol extracts of selected eight ethnomedicinal plants were proved to have no activity against four bacterial strains tested. The tested bacterial strains are found to be resistant to the methanol extracts of plants.

Resistance of some of the bacteria against selected plants have been reported. They were reported by *Gupta et al.*,^[10] while studying the antimicrobial activity of dried alcoholic

extract of *Cymbopogon flexuosus*. The extract is found to be less effective against *E. coli*.

The resistance of *E. coli* and *Klebsiella pneumoniae* against alcoholic extract of *Datura stramonium* was reported by *Gul et al.*,^[9] and *Uma*^[36] respectively.

Detection of bioactive principles from phytochemical screening of ethnomedicinal plant is a new source of therapeutically and industrially valuable compounds that may lead to drug discovery and development. All the secondary metabolites contributes significantly towards the biological activities of medicinal plants such as hypoglycaemic, antidiabetic, antioxidant, antimicrobial, antiinflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc.^[16].

All the ethnomedicinal plants except *Melia dubia* and *Ruta graveolens* were found to possess tannins. Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes^[40].

Flavonoids are present only in *Triumfetta rhomboidea*. It is a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [28, 29]. It also helps in managing diabetes induced oxidative stress. Terpenoids are present in all plants except *Ruta graveolens* and *Triumfetta rhomboidea*. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties [26, 39]. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [18]. Several workers have reported the analgesic [1, 12], antispasmodic and antibacterial properties of alkaloids [34, 20].

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [31]. All the plants except *Atlantia monophylla* and *Melia dubia* possess phenols. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [11].

The extract of *Solanum villosum* revealed the presence of saponins which are known to produce inhibitory effect on inflammation [13]. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness [32, 21].

Steroids present in *Ruta graveolens* and *Triumfetta rhomboidea* help in regulating the immune response [30]. Carbohydrates are macromolecules, and it is composed of water and carbon. All carbohydrates are polar it can be readily converted into glucose which is used as an ultimate source of energy². Glycosides are known to lower the blood pressure according to many reports [19].

The analysis of phytochemicals in methanol extract of *Atlantia monophylla* revealed the presence of tannins, terpenoids and glycosides and absence of alkaloids, flavonoids phenols, phyllotannins, steroids, carbohydrates, saponins and proteins. This is in agreement with findings of Reddy et al., [27].

Phenols, tannins, and terpenoids were found to be present in the methanolic extract of *Cymbopogon flexuosus* and alkaloids, flavonoids, phyllotannins, steroids, carbohydrates, glycosides, saponins and proteins are absent. Avoseh et al., [3] also reported the presence of phenols, tannins, terpenoids and also flavonoids which is not present in the methanolic extract of current study.

The present study reported the presence of phenols, tannins, terpenoids, glycosides and absence of alkaloids, flavonoids, phyllotannins, steroids, carbohydrates, saponins and proteins in *Datura stramonium*. The similar phytochemicals along with alkaloids and flavonoids have been reported by Oyeleke et al., [22]. The analysis by Deshmukh et al., [5] indicated the presence of alkaloids, phenols, carbohydrates, glycosides, terpenoids, steroids and proteins in methanol extract of *Datura stramonium*.

Phytochemical analysis of *Ruta graveolens* indicated the presence of phenols, steroids and glycosides. This is similar to the findings of Pandey et al., [24] and Teklit and Tanveer [35]. Pandey et al., [24] also reported the resistance of *Pseudomonas*

aeruginosa against the methanol extract of *Ruta graveolens* as in the present study.

Melia dubia gave tests for the presence of terpenoids, carbohydrates and glycosides in methanol extract. This is contradictory to the work of Valentina et al., [37] that they have done the phytochemical analysis in different extraction solvents such as n-Hexane extract, petroleum ether extract, acetone extract, ethanol extract and water extract.

The methanol extract of *Solanum villosum* indicated the presence of alkaloids, phenols, tannins, phyllotannins, terpenoids and saponins. This results show the same findings by Venkatesh et al., [38].

Screening for phytochemicals present in *Triumfetta rhomboidea* revealed the presence of alkaloids, flavonoids, phenols, tannins, steroids and glycosides. Similar result was reported by Devmurari et al., [6].

Tests for phenols, tannins, terpenoids and glycosides were found to be positive in the phytochemical screening of methanol extract of *Vitex negundo*. This is similar to the works of Panda and Dutta [23], Srinivas et al., [33], Merlin and Catherine [15], Gautam and Kumar [8], Prasanna and Yuwvarani [25] and Nirmalkumar [17].

Reference

1. Antherden LM. Textbook of Pharmaceutical Chemistry, 8th edn., Oxford University Press, London, 1969, 813-814.
2. Arya V, Thakur N, Kashyap CP. Preliminary Phytochemical Analysis of the extracts of *Psidium* Leaves. J Pharmacogn Phytochem. 2012; 1:1-5.
3. Avoseh O, Opeoluwa O, Pamela R, Benedicta N, Adebol O. *Cymbopogon* Species; Ethnopharmacology, Phytochemistry and the Pharmacological Important Molecules, 2015, 7438-7453.
4. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants. 2015; 4:196.
5. Deshmukh AS, Shelke PD, Palekar KS, Pawar SD, Shinde HS. Antimicrobial Investigation of *Datura stramonium* Leaf Extract against different Microorganisms. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) Ver. I. 2015; 9(9):2319-2399.
6. Devmurari VP, Ghodasara TJ, Jivani NP. Antibacterial Activity and Phytochemical Study of Ethanolic Extract of *Triumfetta rhomboidea* Jacq. International Journal of Pharm Tech Research. 2010; 2(2):1182-186.
7. Dobelis IN. Magic and Medicine of plants. The Reader Digest Association Inc., New York, Montreal, 1993, 8-48.
8. Gautam K, Kumar P. Evaluation of Phytochemical and Antimicrobial study of Extracts of *Vitex negundo* Linn Int. J Drug Dev. & Res. 2012; 4(4):192-199.
9. Gul H, Rubina N, Muhammad AK, Shazia H, Nabila Y. Antibacterial and antifungal activity of different extracts of *Datura stramonium* (branches and leaves sample). E3 Journal of Biotechnology and Pharmaceutical Research. 2012; 3(9):141-148.
10. Gupta AK, Ritam M, Deepak G. A Study on Antimicrobial Activities of Essential Oils of Different Cultivars of Lemongrass (*Cymbopogon flexuosus*). Pharmaceutical Sciences. 2016; 22:164-169.
11. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int. J Mol. Sci., 2007, 950-988.

12. Harborne JB. *Phytochemicals Methods*. Chapman and Hall Ltd., London, 1973, 49-188.
13. Just MJ, Recio MC, Giner RM, Cueller MU, Manez S, Billia AR *et al*. Antiinflammatory activity of unusual lupine saponins from *Bupleurum fruticosens*. 1998; 64:404-407.
14. Karthikeyan A, Shanthi VN. A Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* L. *Int. J Green Pharm*. 2009; 3:78-80.
15. Merlin R, Cathrine L. Preliminary Phytochemical Screening and Antibacterial Activity on *Vitex negundo*. *Int J Curr Pharm Res*. 2011; 3(2):99-101.
16. Negi JS, Singh P, Rawat B. Chemical constituents and biological importance of *Swertia*: A review. *Curr Res Chem*. 2011; 3:1-15.
17. Nirmalkumar N. Pharmacognostic and Phytochemical Analysis of *Vitex negundo*. L. *IIRSET*. 2014; 3(7):14502-14505.
18. Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA. Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*. 1994; 46:753-756.
19. Nyarko AA, Addy ME. Effects of aqueous extract of *Adeniaceae sampeloides* on blood pressure and serum analyte of hypertensive patients. *Phytotherapy Res*. 1990; 4(1):25-28.
20. Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* Linn. Plant parts. *J Sustain. Agric. Environ*. 2004; 6(2):140-147.
21. Okwu DE. Phytochemicals and vitamin content of indigenous species of south-eastern Nigeria. *J Sustain. Agric. Environ*. 2004; 6(1):30-37.
22. Oyeleke SB, Adeyemi HR, Abijogun AS. Phytochemical and Antimicrobial property of *Datura stramonium* on some oral pathogenic microorganisms. *G.J.B.B*. 2015; 4(2):139-144.
23. Panda SK, Dutta SK. Antibacterial activity and phytochemical screening of leaf and bark extracts of *Vitex negundo* from simlilipal biosphere reserve, Orissa. *Journal of medicinal plants research*. 2009; 3(4):294-300.
24. Pandey P, Archana M, Subhadip H. Evaluation of Antimicrobial Activity of *Ruta graveolens* Stem Extracts by Disc Diffusion Method. *Journal of Phytology*. 2011; 3(3):92-95.
25. Prasanna K, Yuwvaranni S. Preliminary Phytochemical Screening and Antibacterial Activity of *Daturs metal* and *Vitex negundo* Against Bacterial Cold Water Disease Causing Organism. *Int J pharm Sci*. 2014; 6(5):230-233.
26. Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. *Breast Cancer Res Treat*. 2009; 115:223-239.
27. Reddy S, Srinivas P, Pallavi P, Suresh A, Praveen V. Screening for Antimicrobial Properties of *Vitex negundo*. From Rural Areas of Warangal dist/A.P. India. *International Journal of Pharma and Bio Sciences*, 2010, 1(4).
28. Rio DA, Obdulio BG, Casfillo J, Marin FG, Ortuno A. Uses and properties of citrus flavonoids. *J Agric Food Chem*. 1997; 45:4505-4515.
29. Salah N, Miller NJ, Pagange G, Tijburg L, Bolwell GP, Rice E *et al*. Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. *Arc. Biochem. Broph*. 1995; 2:339-346.
30. Shah BA, Qazi GN, Taneja SC. Boswellic acids: a group of medicinally important compounds. *Nat Prod Rep*. 2009; 26:72-89.
31. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. *Fod Chem. Toxicol*. 2007; 45:1216-1223.
32. Sodipo OA, Akiniyi JA, Ogunbamusu JU. Studies on certain characteristics of extracts of bark of *Pansinystalia macrucas* (K schemp) picrre Exbeille. *Global J Pure Appl. Sci*. 2000; 6:83-87.
33. Srinivas P, Ram RS, Pallavi P, Suresh A, Praveen V. Screening for Antimicrobial Properties of *Vitex negundo* from Rural Areas of Warangal dist/A.P. India. *International Journal of Pharma and Bio Sciences*, 2010, 1(4).
34. Stray F. *The Natural Guide to Medicinal herbs And Plants*. Tiger Books International, London. 1998, 12-16.
35. Teklit GA, Tanveer MS. Phytochemical Screening and Evaluation of Antibacterial Activity of *Ruta graveolens* L. A Medicinal Plant Grown around Mekelle, Tigray, Ethiopia. *Nat Prod Chem Res*. 2015, 3-6.
36. Uma RB. Antimicrobial Activity of *Datura stramonium* L. And *Tylophora indica* (Burm. f.) Merr. *Pharmacology online*. 2009; 1:1293-1300.
37. Valentina P, Ilango K, Kiruthiga, Parimala MJ. Preliminary Phytochemical Analysis and Biological Screening of Extracts of Leaves of *Melia dubia* Cav. *Int. J Res. Ayurveda Pharm*, 2013, 4(3).
38. Venkatesh R, Kalaivani K, Vidya R. Analysis of phytochemicals and Free Radical Scavenging Activity of *Solanum villosum* (Mill). -A Traditional Medicinal Plant in Southern India. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2014; 3(9):741-755.
39. Wagner KH, Elmadfa I. Biological relevance of terpenoids: Overview focusing on mono-di and tetraterpenes. *Ann Nutr Metab*. 2003; 47:95-106.
40. Yadav M, Sanjukta C, Sharad KG, Geeta W. Preliminary Phytochemical Screening of Six Medicinal Plants Used in Traditional Medicine. *Int J Pharm Pharm Sci*. 2014; 6(5):539-542.