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RESEARCH ARTICLE

Preliminary phytochemical screening of selected Medicinal Plants

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ABSTRACT

Plants derived bioactive compounds have been the focus of recent research due to their health promoting effects. There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Phytochemicals have great potency as antimicrobial agents. The present investigation was carried out to assess the qualitative phytochemical analysis of leaves of five medicinal plants i.e. *Phyllanthus amarus, Clerodendrum viscosum, Ailanthus excelsa, Syzigium cumini* and *Cassia occidentalis* by using polar solvent methanol, non polar hexane and aqueous extract. The phytochemical screening of plant extracts revealed the presence of steroids, Saponin, alkaloids, flavonoids, glycosides, phenolic compounds, tannins, terpenoids and lignin. These phytochemicals have potent antimicrobial efficiency against selected infectious micro-organisms.

KEYWORDS

Phytochemicals, Antimicrobial, Potency, Polar solvent, Non-polar solvent.

INTRODUCTION

For thousands of years mankind is using plant source to alleviate or cure illnesses. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products (Tonthubthimthong *et al*., 2001). In 1985 Farnsworth *et al*., identified 119 secondary plant metabolites which were used as drugs. Out of 255 drugs which are considered as basic and essential by the World Health Organization (WHO), 11% are obtained from plants and a number of synthetic drugs are also obtained from natural precursors. Phytochemicals are known to possess antioxidant (Wong *et al*., 2009), antibacterial (Nair *et al*., 2005), antifungal (Khan and Wassilew, 1987), antidiabetic (Singh and Gupta, 2007; Kumar *et al*., 2008a), anti-inflammatory (Kumar *et al*., 2008b), and radio-protective activity (Jagetia *et al*., 2005), and due to these properties they are largely used for medicinal purpose. The development of drug resistance and the undesirable side effects of certain antibiotics have led to the search for new antimicrobial agents, mainly among plant kingdom, in order to find leads with unique chemical structures which may exert a hitherto unexploited mode of action. Deriving potential benefits from plants has always been a field of speculation for researchers and has formed the basis for development of drugs to treat various diseases. Hence forth, screening of plants for the

presence of natural products and beneficial properties presents a major avenue .The resistance acquired by microbes to the existing antibiotics calls for increased efforts in the development of new antibiotics. Although a number of plants with antimicrobial potential have been identified, great number still remains unidentified. Great range of bioclimatic variation from tropical to alpine brings richness in biological diversity. Many kinds of plants are prevalent in India and a large number of them have been used for antimicrobial assay (Watanabe *et al*, 2005). There is a dire need of extensive studies of medicinal plants found with a special reference to their properties to fight against microbial diseases. Therefore, qualitative phytochemical screening of some medicinal plants like *Ailanthus excelsa* (fam.Simarubaceae), *Cassia occidentalis* (fam. Caeselpiniaceae),*Clerodendrum viscosum* (fam.Verbenaceae),*Phyllanthus amarus* (fam. Euphorbiaceae) and *Syzigium cumini* (fam.Myrtaceae) were done which can give support for their antimicrobial efficiency. The main purpose of the present study was collection and identification of plant materials and screening for presence of various phytochemicals in these medicinal plants.

MATERIALS AND METHODS

Collection of plant material and extraction:

The five medicinal plants *Ailanthus excelsa Clerodendrum viscosum Cassia occidentalis Phyllanthus amarus* and *Syzigium cumini* were collected from surrounding areas of Warora Taluka. Stem and Leaf of these medicinal plants were separated, washed carefully with tap water, rinsed with distilled water, air dried for 1 hour, and shade dried. They were ground in to powder and stored in room temperature. The extract of the samples were prepared by soaking 100gm of dried powder in 200ml of different selected solvents like methanol, n-hexane and water for 12 hours. The extracts were filtered using Whatman filter paper No. 42.

Preliminary phytochemical screening:

The different qualitative chemical tests were performed for establishing the profile of given extracts to detect various phytoconstituents present in them.

Phytochemical screening assays:

Test for Alkaloids

Wagner's Test: To 2-3 ml extract with few drops Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

Dragendorff's Tests: To 2-3 ml extract, add few drops Dragendorff's reagent Formation of orange brown precipitate indicates the presence of alkaloids.

Test for Flavonoids:

Pew's Tests: To 2-3 ml extract, added zinc powder in a test tube, followed by drop wise addition of concentrate HCl. Formation of purple red or cherry colour indicates the presence of flavonoids

Shinoda Tests: - To 2-3 ml extract, few fragments of magnesium metal were added in a test tube, followed by drop wise addition of concentrate HCl. Formation of magenta colour indicated the presence of flavonoids.

Test for Glycosides:

Keller-Kiliani Test: To 2 ml extract, add glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green indicates the presence of glycosides.

Concentrate H_2SO_4 Test: To 5ml extract, add 2ml glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4. Brown ring appears indicates the presence of glycosides.

Test for Phenols:

Ellagic Acid Test: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$solution. The solution turned muddy or Niger brown precipitate occur

Test for Saponin

Foam Test: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam indicated the presence of Saponin.

Haemolysis Tests: - Add leaves extract to one drop of blood placed on glass slide. Hemolytic zone appears.

Test for Sterols:

Liebermann-Burchard Test: Mix 2ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops concentrated H_2SO_4 from the side of the test tube. First red, then blue and finally green colour indicated the presence of sterols.

Salkowski Tests: To 2 ml of extract, add 2ml chloroform and 2 ml concentrated $H_2SO_4$and was shaken well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols.

Test for Tannins:
Gelatin Test: To the extract, gelatin (Gelatin dissolves in warm water immediately) solution was added. Formation of white precipitate indicates the presence of tannins.

Test for carbohydrates:
Molisch test: Treat extract with few drops of alcoholic alpha-naphthol. Add 0.2 ml conc sulphuric acid slowly along the sides of test tube, purple to violet colour ring appears at junction.

Fehling's Test: Fehling A and Fehling B reagents are mixed and few drops of extract is added and boiled. A brick red coloured precipitate of cuprous oxide forms, if reducing sugars present.

RESULTS

The phytochemical analysis of aqueous, methanolic and n-hexane extracts of *Ailanthus excelsa*, *Clerodendrum viscosum, Cassia occidentalis*, *Phyllanthus amarus* and *Syzigium cumini* revealed the presence of phytochemicals in varying proportions (Table1-5). A considerable amount of tannins, phenol, Saponin, proanthocyanidins, reducing sugars, flavonoids, terpenoids, glycosides and steroids were found in the aqueous and methanolic extracts while a very less amount of phytochemicals were found in n-hexane extracts

The presence of alkaloid has seen in methanolic and aqueous extract but absent in hexane extracts. Alkaloids are one of the diverse groups of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase. Carbohydrate which constitutes the major edible part of the plant is present in all the above five medicinal plant extracts. Glycoside is present in varying proportion in methanol, aqueous and hexane extract. Cardiac glycoside has shown strong positive result for methanol and aqueous extract but weak positive results in hexane extract. Flavonoids are also known as vitamin P or natural biological modifiers, mildly present in methanolic extract and strongly present in aqueous where as phenol has shown positive result in alcoholic extract compared to aqueous extract.

A direct relationship has been reported between the levels of phenolic compounds and antioxidant potential of plants. Phenolic compounds exhibit their protective action through various mechanisms like preventing the generation of carcinogens from precursors by acting as blocking agents. The compound which possess large amount of flavonoids has found to have inherent ability to modify the body reactions to allergens, viruses and carcinogens.

The test for tannin has given positive result in all the five medicinal plant extracts. Flavonoid is present almost in all the four extracts. Flavonoids, also referred to as bioflavonoid, are polyphenol antioxidants found naturally in plants. Saponin are completely absent in *C. viscosum* and *C. occidentalis* extracts. The presence of tannin has seen in all the extracts except hexane. .High intake of tannin, the phenolpropyranoids showed reduce in the risk of coronary heart diseases.

Photo plate 1: Selected medicinal plants.

Ailanthus excelsa

Cassia occidentalis

Clerodendrum viscosum

Phyllanthus amarus

Syzigium cumini

Table 1: Qualitative analysis of phytochemicals in leaf extract of *A. excelsa*

Sr.No	Phytochemical Test	Methanol Extract	Hexane Extract	Aqueous Extract
1	Test for Alkaloid	-	-	-
2	Test for Flavonoids	-	-	-
3	Test for Glycosides	++	+	-
4	Test for Phenols	++	-	-
5	Test for Saponin	+	-	+
6	Test for Sterols	-	-	-
7	Test for Tannins	++	-	-
8	Test for Carbohydrates	+	-	+

Strongly Present +++ Present ++ Weekly Present + Absent -

*The given results are statistically significant

Fig. 1: Graphical representation of secondary metabolites distribution in the leaf and stem of *A. excels*

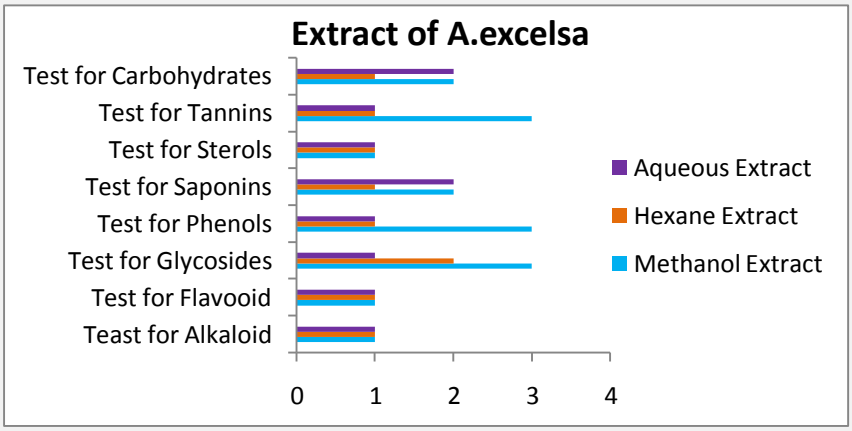


Table 2: Qualitative analysis of phytochemicals in leaf extract of *C. viscosum*

Sr. No	Phytochemical Test	Methanol Extract	Hexane Extract	Aqueous Extract
1	Test for Alkaloid	++	-	++
2	Test for Flavonoid	-	+	+
3	Test for Glycosides	++	+	+
4	Test for Phenols	-	-	-
5	Test for Saponins	-	-	-
6	Test for Sterols	++	+	-
7	Test for Tannins	++	-	+++
8	Test for Carbohydrates	+	+	+

Strongly Present +++ Present ++ Weekly Present + Absent -

*The given results are statistically significant

Fig. 2: Graphical representation of secondary metabolites distribution in the leaf and stem of *C. viscosum*

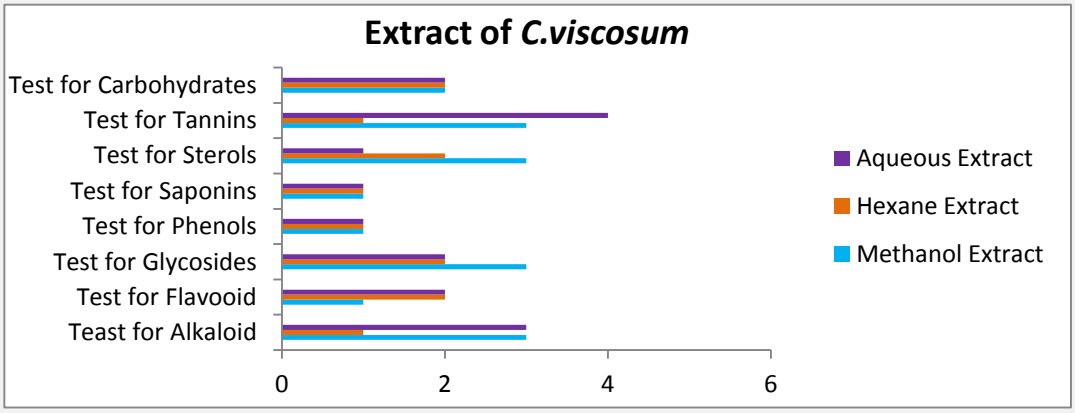


Table 3: Qualitative analysis of phytochemicals in leaf extract of *C. occidentalis*

Sr. No	Phytochemical Test	Methanol Extract	Hexane Extract	Aqueous Extract
1	Test for Alkaloid	++	-	++
2	Test for Flavonoids	-	-	++
3	Test for Glycosides	++	-	++
4	Test for Phenols	++	++	++
5	Test for Saponin	-	-	-
6	Test for Sterols	++	-	+
7	Test for Tannins	+++	-	+++
8	Test for Carbohydrates	++	++	++

Strongly Present +++ Present ++ Weekly Present + Absent -

*The given results are statistically significant

Fig. 3: Graphical representation of secondary metabolites distribution in the leaf and stem of *C.occidentalis*

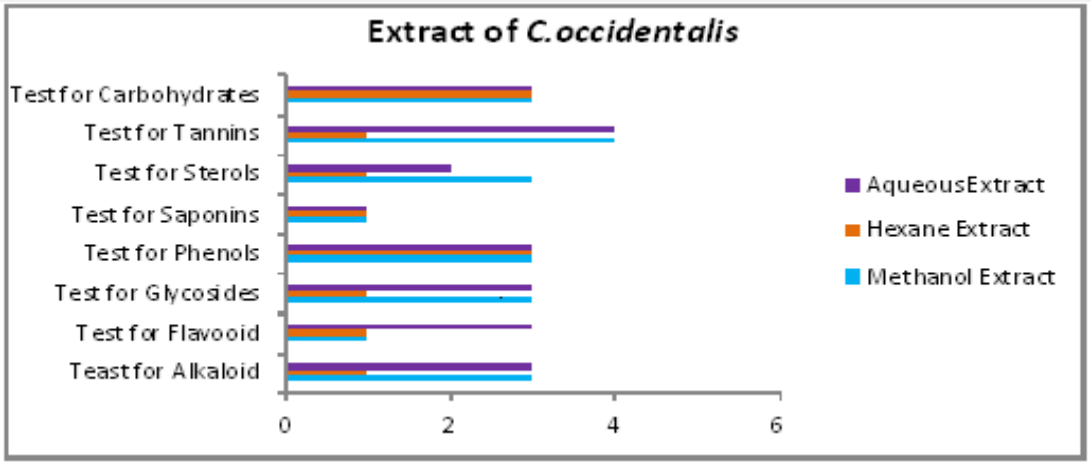


Table 4: Qualitative analysis of phytochemicals in leaf extract of *P. amarus*

Sr. No	Phytochemical Test	Methanol Extract	Hexane Extract	Aqueous Extract
1	Test for Alkaloid	++	-	+
2	Test for Flavonoids	+	-	+
3	Test for Glycosides	+	-	-
4	Test for Phenols	+	-	+
5	Test for Saponin	++	-	++
6	Test for Sterols	++	+++	-
7	Test for Tannins	+	-	+++
8	Test for Carbohydrates	+	_	++

Strongly Present +++ Present ++ Weekly Present + Absent -

*The given results are statistically significant

Fig. 4 : Graphical representation of secondary metabolites distribution in the leaf and stem of *P. amarus*

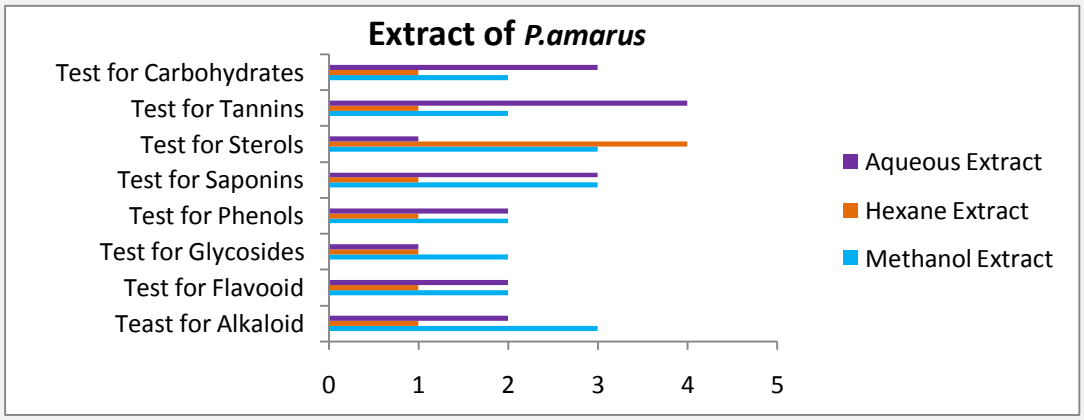


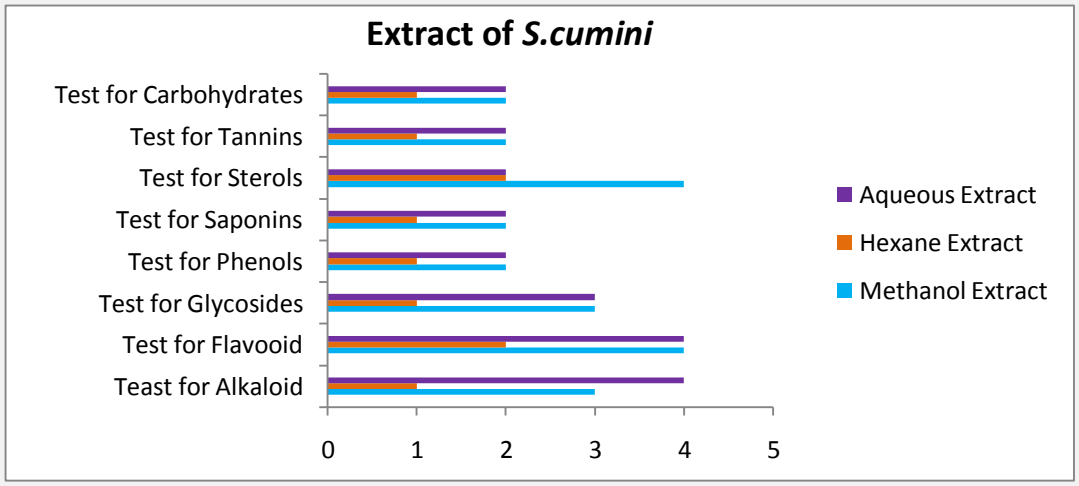
Table 5: Qualitative analysis of phytochemicals in leaf extract of *S. cumini*

Sr. No	**Phytochemical Test**	**Methanol Extract**	**Hexane Extract**	**Aqueous Extract**
1	Test for Alkaloid	++	-	+++
2	Test for Flavonoids	+++	+	+++
3	Test for Glycosides	++	-	++
4	Test for Phenols	+	-	+
5	Test for Saponin	+	-	+
6	Test for Sterols	+++	+	+
7	Test for Tannins	+	-	+
8	Test for Carbohydrates	+	-	+

Strongly Present +++ Present ++ Weekly Present + Absent -

*The given results are statistically significant

Fig. 5: Graphical representation of secondary metabolites distribution in the leaf and stem of *S. cumini*



DISCUSSIONS AND CONCLUSION

Secondary metabolite studies of above five medicinal plants have shown that the presence of carbohydrates, flavonoids, alkaloid, tannin, coumarin, steroid, phenol which are of great importance in the field of drug research. These classes' alkaloids, Saponin, tannins, flavonoids are known to have activity against pathogens and therefore aid the antimicrobial activities of medicinal plants (Ghosh *et al*., 2010). In any research in phytotherapy, it is necessary to choose solvent according to biological activity required and not that which gives a high amount of bioactive compounds. The plants contain triterpenoids shows antimicrobial activity against staphylococcus (Chung *et al*., 2011). Flavonoids are known to inhibit or kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase, protease and also destroy some pathogenic protozoan's. The higher concentrations of more bioactive flavonoids compounds were detected with 70% ethanol due to its higher polarity than pure ethanol (Chantal *et al*., 2005). From the present study it is found that crude extract express good biological capacity which indicates that the substance with powerful biological effect exists in this extract and must be isolated and purified to confirm its pharmacological and medical use.

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