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Asian Journal of Plant Science and Research, 2012, 2 (5):581-587

ISSN : 2249-7412
CODEN (USA): AJPSKY

Preliminary comparative phytochemical screening of root extracts *of Diospyrus ferrea (Wild.)* Bakh and *Aerva lanata* (L.) Juss. Ex Schultes

R. Vijayalakshmi[1*] and R. Ravindhran[2]

[1]*Department of Plant Biology and Plant Biotechnology Ethiraj College for Women, Chennai-8*
[2]*Department of Plant Biology and Biotechnology Loyola College, Chennai-34*

ABSTRACT

Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of compounds. Diospyrus ferrea (Willd.) Bakh is a medicinally important small bonsai tree, belongs to the family Ebenaceae. The root has been used for the treatment of various ailments like dyspepsia and inflammation. Aerva lanata (Amaranthaceae) is erect or prostrate herb, the whole herb is medicinal though the roots are often preferred. It is used in diabetes lithiasis, headache and strangury. To identify and understand the bioactive chemical compounds dry root powders were subjected to different solvents such as hexane chloroform, methanol, ethanol and water sequentially in a soxhlet apparatus. Although, all the five extract exhibited promising phytochemicals, yet maximum phytochemicals was observed in ethanol extract. The hexane and chloroform extract gave positive result for only few secondary metabolites which confirms the less phytoconstituents. The aqueous extract gave positive result for carbohydratre, phytosterol and saponin. The methanol root extract was found to be positive for some of the secondary metabolite such as alkaloids, flavonoids, terpenoids, quinones, tannins, phlobatanins, and reducing sugars. The ethanol root extract of the plant showed the presence of polar and non polar phytoconstituents, hence considered to be suitable solvent for further pharmacological investigation. The current study was carried out to provide requisite phytochemical detail about the root in Diospyrus and Aerva in different type of solvents.

Key words: phytochemicals, *Diospyrus ferrea*, *Aerva lanata*, Root, ethanol extract.

INTRODUCTION

Plants are natural source of producing wide number of phytoconstituents in a most efficient way and with precise selectivity. Since the middle of the 19th centuary different bioactive phytoconstituents have been isolated and characterized. Many of these are used as the active ingredients of the modern medicine or as the lead compounds for new drug discovery. Several plant derived medicine are rich in phenolic compounds such as those used in protection against the coronary heart diseases and carcinogenesis [1&2]. *Diospyros ferrea* is numerically and economically the most important genus of Ebenaceae. The uniqueness of the genus is the elaboration of a large number of pentacyclic triterpenes and juglone based 1, 4-naphthoquinone metabolites. These metabolites can be used as chemical markers for taxonomic studies. Its roots stem, bark and fruits constitute the drug, considered useful in treating the skin disorders [3]**.** Such secondary metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives [4]. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agent with general as well as specific activity [5].

From old days the different *Diospyrus* species are known for their medicinal uses. In many traditional medicinal system of the world a number of *Diospyrus* plants are used as medicinal agents against various diseases. All parts of the plant have been used for medicinal purposes such as the leaves are used for lumbago; the fruits are carminative, astringent and cure biliousness, the seeds and the bark is bitter, astringent and febrifuge[6]. Leaf extract of *Diospyrus khaki* in combination with jasmine is useful in Japan for making anti-tobacco smoking candies. Since the leaves of some *Diospyrus* species example *Diospyrus melanoxylon and Diospyrus tomentosa* are also used for

wrapping bidies. Some of the bioactive compounds such as triterpenoids belonging to the lupine oleanane quinine and ursane series have anti-inflammatory action [7]. *Diospyrus* species in traditional medicinal system of the world are used as anti-fungal and (a) for internal hemorrhage and bed wetting in children. (b) Womens medicine for insomnia and hiccough (c) anti-hypertensive (d) dysponea (e) vermicide and vermifuge (f) sedative (g) antifebrile (h) promote secretion (i) astringents and bactericidal [8]. Triterpenoids have been isolated from *D.lotus* showing anti inflammatory activity [9] Phytochemical studies have been carried out on many *Diospyrus* species have revealed the presence of quinones and napthaquinones derivatives dimeric napthaquinones and lupine triterpenes. These plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties [10]. Medical plants contain large varieties of chemical sub-stances which possess important therapeutic properties that can be utilized in the treatment of human diseases [11]. Microorganism and medicinal plants are rich sources of secondary metabolites, which are potential sources of useful drugs and other useful bioreactive product[12].

Aerva lanata (A. lanata) known as Polpala is a prostrate to decumbent, sometimes erect herb, found throughout tropical India as a common weed in fields and wasteland. Traditionally, *A. lanata* Linn. leaves are used as sap for eye complaints, an infusion is given to cure diarrhea and kidney stone, and root is used in snake bite treatment. The root extract has been given orally for 11 days to degrade the toxic effect induced by the venom toxins. A root decoction is taken orally in empty stomach one day for one month to cure diabetes. The root is diuretic, demulcent, tonic and given to pregnant women. A leaf decoction preparation is used as gargle for treating sore throat and is also used in various complex treatments against guinea worm. A variety of pharmacological activities of this ethnomedicinally important plant has been reported as follows: anthelmintic, demulcent, antiinflammatory, diuretic, expectorant, hepatoprotective and nephroprotective activities [13]. Alcoholic extract of shoots of A. lanata has shown significant antidiabetic and antihyperglycaemic activities in rats. Antimicrobial cytotoxic, urolithiatic, hypoglycemic, antihyperlipidaemic, antiparasitic, antihelmentic activities have also been reported in A. lanata by various workers. Although the preliminary phytochemical studies revealed the presence of various bioactive compounds other than alkaloids, there is no detail study on phytoprofiling of A. lanata root in five different solvents. In India traditional communities like tribal and rural populations are frequently using the crude extracts of local plants for medicinal and other purposes [14]. The present study was undertaken to identify the phytochemicals present in the *Diospyrus ferrea and Aerva lanata* root extract with suitable solvent such as methanol ethanol, chloroform, water and hexane. These herbal drugs contain unique constituents which differs from one herb to another, hence the type and extent of their medicinal property also differs [15].

MATERIALS AND METHODS

Fresh roots of *Diospyrus ferrea and Aerva lanata* were collected from Southern Western Ghats, South India. A voucher specimen (FLOR 24. 144) was deposited in the Herbarium Botanical Survey of India Coimbatore for authentication of plant. After authentification roots were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, methanol, ethanol and aqueous for 48 hrs sequentially in a Soxhlet assembly. The extracts were concentrated, percentage yield calculated and subjected to preliminary phytochemical analysis.

QUALITATIVE METHOD OF PHYTOCHEMICAL SCREENING (Sofowara (1993), Trease and Evans (1989) and Harborne (1973)
The root extracts were analyzed for alkaloids flavanoids, pholabatannins, glycosides, phenols, saponins, lipids and fat, tannins, anthraquinones, quinines, cardiac glycosides, coumarines acids, steroids, phytosterols, proteins carbohydrates etc.

Detection of Alkaloids (Evans, 1997)
About 50 mg of Solvent free extract was stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows:

Mayer's test (Evans, 1997)
To a 1 ml of filtrate, few drops of Mayer's reagent are added by the side of the test tube. The white or creamy precipitate indicated test as positive.

Wagner's test (Wagner, 1993)
To a 1 ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. The color change was observed. A reddish-brown precipitates confirms the test as positive.

Dragendorff's test (Waldi, 1965)
To a 1 ml of filtrate, 2 ml of Dragendorff's reagent are added and the result was observed carefully. A prominent yellow precipitate confirms the test as positive

Detection of Carbohydrate (Ramakrishnan *et al.*, 1994)
Fehlings test:
One ml of extract was boiled on water bath with 1 ml each of Fehling solutions A and B. The color change was observed. A red precipitates indicated presence of sugar.

Barfoed's test
To 1 ml of extract, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. The color change was noted and recorded. A red precipitates indicated presence of sugar.

Benedict's test
To 0.5 ml of extract, 0.5 ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2 minutes and the result was observed. A red precipitates indicated presence of sugar.

Detection Of Glycosides
Legals test
Chloroform (3ml) and ammonia solution (10%) was added to 2ml plant extract. Formation of pink color indicated the presence of glycosides.

Detection of Proteins (Fisher, 1968; Ruthmann, 1970)
The extract was dissolved in 10 ml of distilled water and filtered through Whatmann No.1 filter paper and the filtrate is subjected to tests for proteins and amino acids.

Millon's test (Rasch and Swift, 1960)
To 2 ml of filtrate, few drops of Millon's reagent are added. The result was observed. A white precipitates indicated presences of proteins.

Biuret test (Gahan, 1984)
An aliquot of 2 ml of filtrate was treated with drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. The pink color in ethanol layer indicated presences of proteins.

Detection of amino acid
Ninhydrin test (Yasuma and Ichikawa, 1953)
Two drops of ninhydrin solution (5 mg of ninhydrin in 200 ml of acetone) are added to two ml of aqueous filtrate. The color change was observed. A characteristic purple color indicated the presence of amino acids.

Detection of Phytosterols (Finar, 1986):
Liberdmann-Burchard's test
The extract (5 mg) was dissolved in 2 ml acetic anhydride and one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tube. The formation of blue green color indicated the presence of triterpinoides and phytosteroids.

Detection of Tannins
Ferric chloride test (Mace, 1963)
The extract (5 mg) was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicated the presence of tannins.

Detection of Phenols
Lead acetate test
The extract (5 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitates indicated the presence of phenols.

Detection of flavanoids
An aqueous solution of the extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavanoids.

Detection of coumarins
10% NaOH (1ml) was added to 1 ml of the plant extracts formation of yellow color indicated presence of coumarines.

Detection of Saponin
Distilled water 2ml was added of each plant extracts and shaken in a graduated cylinder for 15 mins lengthwise. Formation of 1cm foam indicates the presence of saponin.

Detection of Quinone
Concentrated sulphuric acid (1ml) was added to 1ml of each of the plant extract. Formation of red color indicated the presence of Quinones.

Detection of Cardiac glycosides
Glacial acetic acid (2ml) and few drops of 5% ferric chloride were added to 0.5% of the extract. This was under layered with 1ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated presence of cardiac glycosides.

Detection of Terpenoid
Chlorofrm (2ml) and concentrated sulphuric acid was added carefully to 0.5 ml of extract. Formation of red brown color at the interface indicated the presence of terpenoids.

Detection of Acids
Plant extract 0.5 ml was treated with sodium bicarbonate solution. Formation of effervescence indicated presence of acids.

Detection of Phlobatannins
Few drops of 10% ammonia solution were added to 0.5 ml of root extract. Appearance of pink color precipitates indicated the presence of phlobatannins.

Detection of Anthraquinones
Few drops of 2% HCL were added to 0.5 ml of root extract. Appearance of red color precipitate indicated presence of anthraquinones.

Detection of steroids and phytosteroids
To 0.5 ml of the plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicated the presence of phytosteroids.

Detection of Fixed oils (Kokate,1999)
A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presences of fixed oil.

Detection of fat
Saponification test
A few drops of 0.5 N alcoholic potassium hydroxide solutions are added to small quantity of extract along with drop of phenolphthalein. The mixture was heated on water bath for 2h. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Detection of Gum and mucilages (Whistler and Be Miller, 1993)
The extract was dissolved in 5 ml of distilled water and to this 25ml of absolute alcohol was added with constant stirring. White or cloudy precipitate indicated the presence of gums and mucilage's.

RESULTS AND DISCUSSION

Phytochemical screening of various extracts of the *Diospyros ferrea and Aerva lanata roots*, fractions showed the presence of most important phytoconstituents. The medicinal value of the title plant can be correlated due to the presence of various bioactive chemical constituents (Table-1 and 2).

Hexane extract- In *Diospyrus* hexane extracts confirms the presence of alkaloid, cadiac glycosides, coumarines and neagative to rest of the phytoconstituents. In *Aerva* extract confirms the presence of saponin, alkaloids, cardiac glycosides and phlobatins.

Chloroform extract- The chloroform extract gave positive results for cardiac glycosides terpenoids, proteins, steroids and phytosterols, anthraquinones. In *Aerva* extractconformstannins, saponin, flavonoids, alkaloids, cardiac glycosides, terpenoids, proteins, steroids and phytosterols.

Methanol extract-The methanol extract of *Diospyrus* gave positive results to carbohydrates, tannins, flavanoids, alkaloids, quinones, terpenoids, triterpenoids, proteins, steroids, phytosterols and negative to rest. In *Aerva* methanolic root extract positive to carbohydrates, tannins, saponon, flavonoids, alkaloids, cardiacglycosides, terpenoids,proteins, steroids,phenols, phytosterols and negative to coumarine and acids.

Ethanol extract- The ethanol extract of the plant showed the presence of polar and non polar phytoconstituents. The ethanol extract confirms the presence of alkaloid, carbohydrate, protein, phytosterol, phenol, tannins, terpenoids triterpenoids, cadiac glycosides, steroids, fat and gum. In *Aerva* ethanol root extract confirms the presence of carbohydrate, protein, phytosterol, phenol, tannins, terpenoids triterpenoids, cadiac glycosides, steroids,and negative to coumarine, acids, and anthraquinones. From the above test the ethanol extract was found to possess the most important phytoconstituents.

Aqueous extract-The aqueous root extract confirms the presence of saponin which is universally absent in the rest. Aqueous extract gave positive results to cardiac glycosides terpenoids, proteins and negative to rest of the phytoconstituents. Glycosides and resins are found to absent in all the extracts. In *Aerva* extract confirms the presence of carbohydrate, protein, phytosterol, phenol, tannins, terpenoids triterpenoids, cadiac glycosides, steroids,and negative to coumarine, acids, anthraquinones, steroids.

In the present study *Diospyrus* and *Aerva* crude ethanol extract contains all the polar and non polar components. The presence of quinones, terpenoids and steroids, triterpinodes, phytosterol, saponin in the roots has not been reported before (Table-1, 2). The hexane and chloroform extract are positive only for few metabolite compare to the other extract. The literature revealed that the genus *Diospyros* have pesticide and biological activities [16]. The specific activity of the *Diospyros* may be attributed to the presence of quinones, terpenoids, steroids and phenolic compounds which need further investigation.

Table1.Qualitative phytochemical analysis of *Diospyrus ferrea* root extract

Pytochemical test	Hexane extract	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Carbohydrates	-	-	+	+	_
Tannin test	-	-	++	+	-
Saponin	-	-	-	-	+
Flavanoid	-	-	++	++	-
Alkaloid	+	-	++	++	-
Quinones	-	-	+	++	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	++	-	++	+
Terpenoid test	-	+	+	++	+
Triterpenoids	-	-	+	++	-
Phenols	-	-	+	++	-
Coumarins	+	-	-	-	-
Acids	-	-	-	-	-
Proteins	-	+	+	+	+
Steroids	-	+	+	++	-
Phytosterols	-	+	+	+	-
Anthraquinones	-	+	-	-	-
Phlobatannins	-	-	-	-	-

The yield of the extract was found to be more in ethanol and methanol compare to the rest of the solvent. Small amount of extract was obtained from hexane and chloroform as compared to methanol and ethanol extract. The percentage of the yield in *Diospyru*s was recorded for each extract were found to be more in ethanol (5.2%) followed by methanol (3.6 %) and water (0.67%). The least amount of the yield was recorded for hexane (0.4%) and chloroform (0.61%) (Table-3). The percentage of the yield in *Aerva* was recorded for each extract were found to be more in ethanol (0.7%) followed by methanol (0.5 %) and water (0.4%). The least amount of the yield was recorded for hexane (0.5%) and chloroform (0.4%)

The phytochemical screening and quantitative estimation of the percentage crude yields the most promising secondary metabolites such as alkaloids, flavonoids, phenol, proteins, aminoacids tannin, and carbohydrates. They were known to show the medicinal activity as well as physiological activity [17]. Alkaloid, tannins, terpenoid, flavonoid and phenol are found abundant in the ethanol root samples. It should be noted that phenol components are of importance and interest in pharmacy due to their relationship with cancer activity [18, 19].

The presence of such components alkaloid, quinones, terpenoids, tannin phytosterol, coumarine quinones and steroids in ethanol root may have good anti-inflammatory activities, anti cancer activity but no work have been reported so far in anticancer activity which needs further investigation.

Table2.Qualitative phytochemical analysis of *Aerva lanata* root extract

Pytochemical test	Hexane extract	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Carbohydrates	-	-	+	+	+
Tannin test	-	+	++	+	-
Saponin	+	+	+	+	+
Flavanoid	-	+	++	++	-
Alkaloid	+	+	++	++	+
Quinones	-	-	+	++	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	++	-	++	+
Terpenoid test	-	+	+	++	+
Triterpenoids	-	-	+	++	+
Phenols	-	-	+	++	+
Coumarins	-	-	-	-	-
Acids	-	-	-	-	-
Proteins	-	+	+	+	+
Steroids	-	+	+	++	-
Phytosterols	-	+	+	+	+
Anthraquinones	-	-	-	-	-
Phlobatannins	+	-	+	+	-

Table 3. Yield of root extract of *Diospyros ferrea and Aerva lanata*

Parts	Methanol	Ethanol	Aqueous	chloroform	Hexane
D.ferrea root	3.6%	5.2%	0.67%	0.61%	0.4%
A.lanata root	0.5%	0.7%	0.4%	0.4%	0.5%

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins and steroids in different root extracts of *Diospyrus ferrea*. Some of the phyto chemical such as phlobatinis,saponin,tanninsand terpenoids are specific to *Aerva* .These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmaological actions[20]. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides, terpenoid quinones, and alkaloids. According to Rath et al., (2009), acetone and ethanolic bark extract revealed the most of the phytoconstituents[21]. The presence of such phytoconstituents may be correlated with the fact that ethanol extract showed maximum activity against the bacterial strains in *D.melanoxylan*. On contrary, Sunitha Singh (2012) proved most of the phytoconstituents in chloroform root extract of *Diospyrus kaki*. The extract also revealed the presence of plumbagin, naptha quinine and isodiospyrin. These phytoconstituents may be responsible for the many pharmacological actions of the plant like wound healing cholesterol lowering and antidiabetic activity [22]. Yamuna et al., observed the steroid and triterpenoids presence in the stem, leaves, root, flower and seeds of *A. lanata* and confirmed it in HPTLC [23]. Further work could also be possible to investigate the specific phytoconstituents responsible for these activities.

CONCLUSION

The tested ethanolic root extract of *Diospyrus ferrea and Aerva lanata* showed some of their chemical components such as flavonoids, alkaloids saponins, tannins and coumarines, quinones and terpenoids. There is no doubt that these plants are reservoir of potentially useful chemical compounds which serve as drugs, provide newer leads and clues for modern drug design. Due to its many medicinal properties there is enormous scope of future research on *Diospyrus ferrea* and *Aerva lanata* further clinical and pharmacological study should be conducted to investigate the unexploited potential of this plants.

Acknowledgements
My sincere thanks to Dr.ShyamalaKanakarajan, HOD, Dept. of Plant Biology and Plant Biotechnology, Ethiraj College for Women, for her support and providing lab facilities.

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