

Phytochemical evaluation of *Eucalyptus citriodora* Hook. and *Lawsonia inermis* Linn

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ABSTRACT

Use of plant based drugs for curing various diseases is as old as human civilization. Plant based medicines are the base of many of the modern pharmaceuticals we used today. The aim of the study was to find out the active constituents of Petroleum ether, chloroform, benzene, acetone, methanol and aqueous leaf and seed extract of *Eucalyptus citriodora* and *Lawsonia inermis*. A qualitative phytochemical analysis was performed for the detection of alkaloids, flavonoids, steroids, carbohydrates, saponins, tannins and volatile oils. Flavonoids, carbohydrates and tannins present in petroleum ether, acetone extract of *Lawsonia inermis* leaf. Chloroform and acetone extract shows the presence of volatile oils whereas steroids were found only in acetone and methanol extract of *L. inermis* leaf. Alkaloids are totally absent in all leaf as well as seed extract of *L. inermis*. Tannins were found to be present in all extracts of *Eucalyptus citriodora*. Flavonoids, steroids and volatile oils are present in almost all extracts of *E. citriodora* leaf and seeds.

Key words: *Lawsonia inermis*, *Eucalyptus citriodora*, Percent extractive value, Phytochemical evaluation.

INTRODUCTION

Higher plants are untapped reservoirs of various valuable chemicals and contain a wide spectrum of secondary metabolites viz. phenols, flavonoids, quinines, essential oils, alkaloids and sterols etc. which are responsible for antimicrobial activity of herbal medicines (Sandu and Arora, 2000). Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In the Indian system of medicine most practitioners formulate and dispense their own recipes. They use various formulations of plant extracts for treatment of various ailments.

Antimicrobial screening is usually done with crude alcoholic or aqueous extract prepared either by cold or hot extraction method. For cold

extraction, dried powdered plant material is suspended in cold solvent for 24-48 hours. After which decoction is filtered and solvent is evaporated. Dried residue is used as crude extract. Crude extracts prepared by this method have been screened for antimicrobial activity by several workers (Ballal *et al.*, 2001; Singh and Majumdar, 2001; Mamatha *et al.*, 2004). Hot extraction process involves boiling of dried plant material with the solvent in Soxhlet assembly till complete extraction takes place. In this process, single solvent or a series of solvents ranging from polar to non-polar can be used (Harborne, 1984; Kokate *et al.*, 1990). Combination of different solvent ensures complete extraction of all kinds of primary as well secondary metabolites present in the plant or its parts depending on the solvents used. Petroleum ether, Chloroform, Ethyl acetate, Ethanol or Methanol and Hydroalcohol has been used by several workers for

successive extraction of active compounds in plant extract (Chakraborty and Patil, 1997, Balkrishnan *et al.*, 2003).

Each plant species of this universe has its own specific set of secondary metabolites. Combined knowledge of biological activity and chemical constituents of the plant desirable for discovery of new class of compounds. Each fraction prepared by successive extraction carries a specific set of secondary metabolites because solubility of secondary metabolites differs with different solvent. Qualitative phytochemical tests are used to detect phyto-constituents present in individual fraction. Several workers have studied phytochemical properties of plant extracts by qualitative phytochemical tests. Khadikar *et al.*, (2001) investigated phytochemical properties of water extract of *Boerhaavia diffusa* and *Azadirachta indica*. Various fractions of *Argemone mexicana* Linn. were subjected to phytochemical tests by Patil *et al.*, (2001). Ajali *et al.*, (2002) reported presence of secondary metabolites in various fractions of *Euphorbia poissoni* stem bark extract.

Eucalyptus citridora is an evergreen tree of family myrtaceae with lemon scented leaves which medicinal values as powerful antiseptic, releasing cough and cold are known from a long time. *Lawsonia inermis* is an herb of family Lythraceae commonly used as drug in common peoples.

In the present study, various organic solvent extracts of *Eucalyptus citridora* and *Lawsonia inermis* were prepared by hot extraction methods. In the next step, the extract was subjected for phytochemical analysis by qualitative phytochemical tests.

MATERIAL AND METHODS

Plant material

The leaves and seeds of *Lawsonia inermis* and *Eucalyptus citridora* were collected from campus of University College of science, Udaipur. The plants were identified in Department of Botany, Rajasthan University, and was given a voucher specimen no. RUBL 20427 (*Lawsonia inermis*) and RUBL 21256 (*Eucalyptus citridora*) which was shade

dried at room temperature. The leaves were cut into small pieces and than seeds and leaves were finely grounded in an electric grinder. The ground material was passed through sieve no. 240 so as to obtain a powder of 60 mesh size, which was used for extract preparation. Hot extraction procedures were followed to obtain various extracts.

Preparation of extract

Reflux method of solvent extraction was used for successive extraction of different organic constituents of dried and powdered leaf as well as seeds. (Harborne, 1984, Kokate *et al.*, 1990). Solvent series for successive separation was as follows:

Pet. Ether →Benzene→Chloroform→Acetone→Methanol→Water

This method involves continuous extraction of powdered dried plant material in soxhlet apparatus with a series of organic solvents. 40 gm of dry plant powder was kept in soxhelt extraction unit and extracted with 280 ml of solvent. The process was repeated till complete extraction took place. Extracted plant material was vacuum dried and placed in hot air drier. Dried extract was stored in air-tight jar, kept in refrigerator.

Percent extractive value

The dried extract and fractions were weighed and their percentage in terms of the dry weight of the plant material was estimated by the following formula and given in table 1 and 2.

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

Phytochemical study of various leaf and seed extracts of *L. inermis* and *E. citridora*

All solvent extracts prepared was subjected for the identification of different secondary metabolites by qualitative tests according to standard methods (Kokate *et al.*, 1990).

Alkaloids

Alkaloids are compounds having one or more nitrogen containing heterocyclic ring. Presence of alkaloids in methanolic extract was

tested by performing Wagner's test. Small amount of extract was taken in a test tube and few drops of dilute HCl was added to it and filtered. Wagner's reagent was added to filtrate. Development of red colour indicated presence of alkaloids.

Carbohydrate / Glycosides

Carbohydrates are widely distributed in plants and can be detected by Fehling test. Small amount of extract was dissolved in 5 ml distilled water and filtered. The filtrate was collected. The filtrate was taken and few drops of equal quantity of Fehling A and Fehling B solution were added and heated. Development of brick-red colour indicates presence of carbohydrate.

Tannins

Chemically tannins contain the mixture of complex organic substances in which polyphenols are present. Ferric chloride test used to detect presence of tannins. Extract was mixed with ferric chloride and lead acetate. Development of white precipitate indicated presence of tannins.

Saponins

Saponins are complex glycosidal compounds in which the aglycone is tri-terpenoid or steroidal in nature. Foam test was used to detect presence of saponins. Small of extract was taken in a test tube and 20 ml distilled water was added to it. Then it was shaken for 15 minutes. Formation of layer of foam at surface indicated presence of saponins.

Flavonoids

Flavonoids usually occur in plants as glycosides in which one or more of phenolic hydroxyl groups are combined with sugar residues. Extract was taken in a test tube and concentrated H_2SO_4 was added to it. Development of yellow-orange colour indicated presence of flavonoids.

Phytosterols

Sterols are tri-terpenes, which are based on cyclopentane perhydroxy phenanthrene ring system. They are also called as Phytosterols. Libermann's and Burchard's test was used for detection of Phytosterols. Small amount of extract was taken in a test tube and alcoholic KOH added to it. Saponification takes place, which was diluted

with distilled water and ether. This ethral extract was evaporated and residue was collected. Libermann's and Burchard's test was done with this residue. The residue was mixed with 10 ml of acetic anhydride and 10 ml of concentrated H_2SO_4 under room temperature.

Volatile Oils

The odorous volatile principles of plants are known as volatile or essential oils. Sudan III test was used to detect presence of volatile oils. Small amount of extract was taken in a test tube and alcoholic Sudan III added to it. Development of red colour indicated presence of volatile oils

RESULTS

Results obtained in extraction and phytochemical analysis is given in table 1-6. Percent extractives of crude extracts of leaf and seed of *Lawsonia inermis* are given in table 1. Higher percent extractive value was obtained with 100% methanol extract of leaf i.e. 18.62%. 6.9%, 6.45%, 4.3% and 3.52% extract yield were obtained using 100% Petroleum ether, chloroform, aqueous, acetone leaf extract. Methanolic and acetone extract of seed gave 5.25% and 4.85% extractive value respectively. Benzene fraction of leaf and chloroform fraction of seed gave minimum extract percentage value 0.67% and 0.87% respectively.

Table 2 shows percent extractive values of *Eucalyptus citridora* leaves and seed extract obtained with different organic solvents. Methanol fraction of leaf and seed gave maximum percent extractive value i.e. 15.85% and 5.8%, respectively. Minimum percentage of extract yield was obtained with benzene and acetone fraction of seed.

Results of phytochemical testing of *Lawsonia inermis* leaves and seed extract obtained in different solvents are given in table 3 and 4. Tannins, carbohydrate, saponins and flavonoids were found to be present in petroleum ether fraction of *L. inermis* leaf. Benzene and chloroform fraction of leaf exhibited presence of flavonoids and tannins. Steroids, saponins, volatile oils, tannins and flavonoids were found to be present in acetone extract of *L. inermis* leaf. Methanol fraction of leaf showed positive reaction for steroids, tannins,

carbohydrates, saponins and flavonoids whereas aqueous fraction exhibited the presence of tannins, carbohydrates and steroids.

Both petroleum ether and acetone fraction of *L. inermis* seed showed presence of alkaloids, steroids and flavonoids. Chloroform extract exhibited positive reaction for alkaloids, steroids, tannins and

flavonoids. Methanol and aqueous fraction gave positive results for steroids and tannins.

Phytochemicals observed in *Eucalyptus citridora* leaf and seed extract are present in table 5 and 6. Tannins are present in all leaf and seed fractions. Petroleum ether fraction of leaf shows the presence of steroids, tannins and flavonoids.

Table 1: Percent Extractive of different fractions of *Lawsonia inermis* Leaf and Seed Extract

S. No	Solvent	Percent Extractive Value	
		Leaf	Seed
1.	PE fraction	6.9	3.97
2.	Benzene fraction	0.67	1.55
3.	Chloroform fraction	6.45	0.87
4.	Acetone fraction	3.52	4.85
5.	Methanol fraction	18.62	5.25
6.	Aqueous fraction	4.3	2.02

Table 2: Percent Extractive of different fractions of *Eucalyptus citridora* Leaf and Seed Extract

S. No	Solvent	Percent Extractive Value	
		Leaf	Seed
1.	PE fraction	6.45	3.4
2.	Benzene fraction	2.87	1.2
3.	Chloroform fraction	3.52	2.8
4.	Acetone fraction	5.82	1.6
5.	Methanol fraction	15.85	5.68
6.	Aqueous fraction	4.3	4.2

Table 3: Phytochemical Screening of Various Fractions of *Lawsonia inermis* Leaf Extract

Fractions	Alkaloid	Steroid	Volatile oils	Fat	Tannins	Carbohydrate	Saponin	Flavonoid
PE	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Benzene	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
Chloroform	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve
Acetone	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
Methanol	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve
Aqueous	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve

+ve = Presence -ve = Absence

Table 4: Phytochemical screening of various fractions of *Lawsonia inermis* seed extract

Fractions	Alkaloid	Steroid	Volatile oils	Fat	Tannins	Carbohydrate	Saponin	Flavonoid
PE	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve
Benzene	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve
Chloroform	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
Acetone	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
Methanol	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve
Aqueous	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve

+ve =Presence -ve = Absence

Table 5: Phytochemical screening of various fractions of *Eucalyptus citridora* leaf extract

Fractions	Alkaloid	Steroid	Volatile oils	Fat	Tannins	Carbohydrate	Saponin	Flavonoid
PE	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
Benzene	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve
Chloroform	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
Acetone	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve
Methanol	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve
Aqueous	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve

+ve =Presence -ve = Absence

Table 6: Phytochemical screening of various fractions of *Eucalyptus citridora* seed extract

Fractions	Alkaloid	Steroid	Volatile oils	Fat	Tannins	Carbohydrate	Saponin	Flavonoid
PE	-ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Benzene	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve
Chloroform	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
Acetone	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve
Methanol	-ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve
Aqueous	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve

+ve =Presence -ve = Absence

Steroids and flavonoids are also present in almost all fractions but alkaloids are found in only chloroform extract of *E. citridora* leaf.

DISCUSSION

Medicinal plants are source of great economic value in the Indian subcontinent.

Medicinal value of plants has been related to their enormous potential to synthesize several chemical substances by the secondary metabolism which are complex in structure. These metabolites include tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and organic acids (Nychas, 1996). Plant synthesizes these secondary metabolites for defence against attack by insects,

herbivores and microorganisms (Fiori *et al.* 2000). These compounds are not necessarily involved in the essential metabolism of cells but are responsible for the colors, flavour and the fragrance of the plant. Hence, it is necessary to identify the phytochemical constituents present in plant extracts. Investigation into antimicrobial activities of plant exposes the plants as potential source of therapeutic agents.

The solubility of different metabolites differs with different solvents and to find out which group of compounds is responsible for antimicrobial activity. Since most of the components responsible for antimicrobial activity are aromatic and saturated organic compounds, they are usually extracted with methanol (Taylor *et al.* 1996; Silva *et al.* 1997).

Eloff (1998) found that extracts prepared in initial solvent do not demonstrate greatest activity and therefore examined variety of solvents for their ability to extract antimicrobial compounds from plants. He reported that anthocyanins, starch, tannins, saponins, polypeptides and reducing sugars are soluble in water where as terpenoids, flavonoids, alkaloids, fatty acids and coumarins are soluble in organic solvents. Similar findings have been reported by several workers (Scalbert, 1991; Mendoza *et al.* 1997). Tannins and reducing sugars are soluble in both water as well as organic solvents but their solubility is more in organic solvents as compared to water (Cowan, 1999).

Harborne (1984), Kokate *et al.* (1990) and Cowan (1999) suggested that successive extraction of plant secondary metabolites should be done in petroleum ether followed by benzene, chloroform,

acetone, alcohol and finally with water i.e. from non polar to polar solvents. Extraction of secondary metabolites from plant material by hot extraction with petroleum ether separates sterols, waxes and fatty acids leaving behind residue containing the defatted plant materials. Subsequent extraction of this residue with benzene separates out sterols and flavonoids. Terpenoids and flavonoids get extracted with chloroform. The last solvent i.e. alcohol removes alkaloids, flavonoids, polyphenols, tannins and reducing sugars from the residue. Finally extraction with water yields remaining water soluble metabolites such as anthocyanins, starch, tannins, saponins and polypeptides. (Zhang and Lewis, 1997; Scalbert, 1991).

Phytochemical tests reveal the broad category or group of secondary metabolites present in the plant extracts. Results of phytochemical testing of different fractions show the presence of steroids, volatile oil, tannins, carbohydrate and flavonoids in acetone fraction of *L. inermis*. leaf. Aqueous fraction of *L. inermis* seed shows presence of steroids and tannins. Eucalyptus leaf and seed shows are abundant in tannins, steroids, volatile oils and flavonoids. Several workers have studied phytochemical property of plant extracts by qualitative method. (Okore *et al.* 2007; Ayandele and Adebisi, 2007).

Thus phytochemical study reveals a group of secondary metabolites present in the plant extracts which have been considered to be a potential source of antimicrobial and any other biological activity of plant.

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