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CHARACTERIZATION OF PHYTOCONSTITUENTS IN LEAF EXTRACTS OF WEEDS

SHAMEEMBANU A. BYADGI* AND SADHANA D. KULLOLI
Department of Textile and Apparel Designing,
College of Rural Home Science, University of Agricultural Sciences, Dharwad - 580 005, Karnataka
e-mail: shama29@rediffmail.com

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***Corresponding author**

ABSTRACT
The objective of the study was to screen the presence of phytoconstituents in different leaf extracts of *Achyranthes aspera*, *Cassia serecia* and *Mimosa pudica* weeds both qualitatively and quantitatively. Solvents such as ethanol, methanol and distilled water were used for extraction. Phytochemical screening was carried out using standard test methods. Total phenolic content (TPC) was determined by Folin-Ciocalteu reagent assay method. Results revealed that the yield of all the leaf extracts *i.e.*, *A. aspera* (40 ml), *C. serecia* (40 ml) and *M. pudica* (43 ml) was found to be high in ethanol and methanol solvents compared to respective distilled water extracts. The phytochemical screening revealed that alkaloids, flavonoids, phenolics & tannins and terpenoids were found in all the leaf extracts of selected weeds. However, saponins were present in all the leaf extracts of *A. aspera*; ethanol and methanol extracts of *M. pudica* but absent in *C. serecia* leaf extacts. Further, ethanolic extract of *C. serecia* and *M. pudica* exhibited maximum TPC while *A. aspera* depicted higher amount of TPC in methanolic extract. It can be concluded that the selected weeds can be used as potential ingredient in pharmaceutical and neutraceutical industries for preparing novel drugs.

INTRODUCTION

Plants are used in different countries as a source of many potential and powerful drugs. Over 50 per cent of all modern clinical drugs are of natural product origin that plays an important role in the pharmaceutical industries (Veerachari and Bopaiah, 2012). Traditional medicine using plant extracts continue to provide health coverage for over 80 per cent of the world's population, especially in the developing world (Pranoothi *et al*., 2014).

In India many unwanted plants so called weeds are very common dominant and wide spread in the various crop fields. Weeds constitute about 250 species which are prominent in agricultural and non-agricultural system; out of which about 20% weeds could be profitably exploited for both domestic and export purposes. They can be used for feed, fodder, green manure, medicinal, biomass based energy, soil conservation and other purposes (Giri and Dhanalakshmi, 2015). The phytochemicals present in the weeds serve as the great reservoirs of many new and potential drugs. Screenings for biological activity using simple bioassays have now been added to give a better identification of the usefulness of weeds (Dhole *et al*., 2012).

Achyranthes aspera is an important medicinal herb found as a weed throughout India. *A. aspera* belongs to family Amaranthaceae commonly known as *Latjeera* (Hindi) & Rough Chaff tree (English). It is an erect or procumbent, annual or perennial herb, often with a woody base, commonly found as a weed of waysides, on roadsides (Srivastav *et al*., 2011). Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts used medicinally. The plant possesses activities like diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and various other important medicinal properties. The crushed plant is used in pneumonia and infusion of the root is used as mild astringent in bowel complaints.

Cassia serecia is a shrub that belongs to Caesalpinaceae family. It is a common weed that grows in waste lands and along the roadsides. *C. serecia* is commonly known as One leaf Senna (English), *Chogache* (Kannada) and *Chakvat* (Hindi). The leaves are used as vegetable, fried with other vegetables like brinjal, potato, etc. Decoction of vegetables and mature leaves is used as a laxative useful in curing ring-worm and skin diseases. *C. serecia* is one of the promising plant in suppressing the growth of parthenium (Veerachari and Bopaiah, 2012).

Mimosa pudica belongs to the taxonomic group Magnoliopsida and family Mimosaseae. The species is native to South America and Central America (Varnika *et al*., 2012). *M. pudica* commonly known as 'Touch me not' in English and '*chui-mui*' in Hindi is a creeping perennial herb found in waste lands, lawns, pastures and along road side. The plant has been described as "*sparshaat sankochataan yaati punashcha prasruta bhavet*" – a plant which folds itself when touched and spreads its leaves after a while. The roots, leaves and flower heads of *M. pudica* are used in Ayurveda. The plant is commonly used for bleeding disorders like menorrhagia, dysentry with blood, mucus and piles. It is mainly used in herbal preparation for gynaecological disorders. All five parts of the plant – leaves, flowers, stem, roots and fruits (*i.e.*, *panchang*) are used as medicines in

traditional health care system.

The metabolites of plant are commercially important and find its use as raw material for various scientific investigations and in number of pharmaceutical compounds. In recent times, the blind dependence on synthetic drugs is surpassed over to the fact that the herbal drugs are cost effective, easily available and most importantly, with negligible side effects (Sharma *et al*., 2010).

Phytochemical investigations of crude plant extracts depict the presence of active principles in the plant parts like bark, leaves, flowers, roots, fruits, seeds etc. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Veerachari and Bopaiah, 2012). The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant (Pranoothi *et al*., 2014). Secondary metabolites are synthesized in a specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products (steroids, quinines, alkaloids, terpenoids and flavonoids) which are used in drug manufacture by the pharmaceutical industries.

Hence, the present study was carried out with an objective to screen the presence of phytochemicals in leaf extracts of *Achyranthes aspera, Cassia serecia* and *Mimosa pudica* weeds and assess the total phenolic content.

MATERIALS AND METHODS

Plant sources

Fresh leaves of *Achyranthes aspera, Cassia serecia* and *Mimosa pudica* weeds were collected from the premises of University of Agricultural Sciences, Dharwad, Karnataka state (India).

Chemicals

Ammonia, chloroform, ethanol, methanol, hydrochloric acid, sulphuric acid, ferric chloride, sodium hydroxide, sodium chloride, sodium carbonate, lead acetate, gelatin were purchased from Rankem chemicals, Bangalore which were of AR grade. Sodium nitrite and aluminium chloride were purchased from Thomas-Baker, Mumbai. Folin-Ciocalteu and Dragendorff's reagent was purchased from Merck, Germany. Gallic acid standard was procured from Sigmaaldrich, Germany.

Herbal extraction

The matured leaves of the selected weeds were collected, cleaned with distilled water and shade dried at room temperature to remove the traces of moisture. Leaves were crushed to fine powder using mechanical grinder. The dry leaf powder was weighed and mixed with 25 ml of each solvent (ethanol, methanol and distilled water) separately. The extracts were incubated for 24 hours at room temperature. The extracts were centrifuged at 5000 rpm at room temperature and the supernatants were separated. Residue was re-extracted with 25 ml of the respective solvent and the process was repeated (Singh *et al*., 2015). The supernatants obtained were pooled and the extracts obtained were measured and filtered using Whatman filter paper No. 40 (125 mm).

Phytochemical screening

Phytochemical screening is one of the techniques to identify new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolics, steroids, tannins, saponins, *etc* present in the plant extracts. The screening was carried out using standard test methods.

Test for alkaloids

Dragendorff's test

Few drops of Dragendorff's reagent were added to 1 ml of the extract. A prominent yellow precipitate indicates the positive test (Raaman, 2006).

Wagner's test

Few drops of Wagner's reagent were added by the side of the test tube to 1 ml of extract. A reddish-brown precipitate confirms the test as positive (Raaman, 2006).

Test for flavonoids

Ammonia test

A few drops of 1 per cent ammonia solution was added to 1 ml of the extract in a test tube. A yellow colouration was observed for the presence of flavonoids (Rahul *et al*., 2010).

Sodium hydroxide test

Few drops of 20 per cent sodium hydroxide solution was added to 1 ml of the extract. The yellow colour of the extract turns to a colourless solution on addition of hydrochloric acid that depicted the presence of flavonoids (Ajayi *et al*., 2011).

Test for tannins and phenolic compounds

Ferric chloride test

1 ml of the extract was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5 per cent ferric chloride solution was added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of tannins (Raaman, 2006).

Gelatin test

2 ml of 1 per cent solution of gelatin containing 10 per cent sodium chloride was added to 1 ml of the extract. White precipitate indicates the presence of phenolic compounds (Rahul *et al*., 2010).

Lead acetate test

3 ml of 10 per cent lead acetate solution was added to 1 ml of the extract. Appearance of bulky white precipitate confirms the presence of phenolic compounds (Raaman, 2006).

Test for saponins

Foam test

About 1 mL of the sample extract was boiled in 20 ml of distilled water in a water bath and filtered; 5 ml of distilled water was added to 10 ml of the filtrate and mixed vigorously for 15 min to form a stable persistent froth. The presence of froth after 5 min was taken as an indication of presence of saponins (Ajayi *et al*., 2011).

Test for terpenoids

Salkowski test

1 ml of each extract was mixed with 0.5 ml of chloroform and

1 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface formed to show positive results for the presence of terpenoids (Ajayi *et al*., 2011).

Total phenolic content (TPC)

Total phenolic content in the extracts was determined by Folin-Ciocalteu assay method using gallic acid as the reference standard. All the solvent extracts were diluted to appropriate volumes and were mixed with 2 ml of 10 per cent sodium bicarbonate solution, incubated at room temperature for 3 min, later 100 μl of Folin-Ciocalteu reagent was added to the mixture (Jyoti *et al*., 2016). The resulting solution was incubated for 90 min at room temperature under dark, the absorbance was measured at 765 nm using the UV-Vis Spectrophotometer. The TPC was expressed as gallic acid equivalent (GAE) in milligrams per gram of dry leaf.

RESULTS AND DISCUSSION

Yield of extracts

The yield of leaf extracts obtained using ethanol, methanol and distilled water is presented in Figure 1. The yield of *A. aspera* using ethanol and methanol solvents (40 ml /50 ml of solvent) was found to be higher than distilled water (30 ml /50 ml of solvent). Similarly *C. serecia* (40 ml) and *M. pudica* (43 ml) also exhibited higher yield in ethanol and methanol as compared to distilled water (36 ml - *C. serecia* and 37 ml - *M. pudica*) extracts.

Phytochemical screening

Table 1 records the phytochemical screening of the extracts of selected species. It is observed from the Table that all the extracts of *A. aspera, C. serecia* and *M. pudica* exhibited positive results for alkaloids proved by Dragendorff's and Wagner's tests. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is cytotoxicity and have reported to possess analgesic, antispasmodic and antibacterial properties (Yadav and Agarwala, 2011).

The presence of flavonoids was positively proved by both tests (ammonia and sodium hydroxide) in all the extracts of *A. aspera, C. serecia* and *M. pudica*. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and have been found to be antimicrobial substances against wide array of microorganisms in vitro (Yadav and Agarwala, 2011).

All the leaf extracts (ethanol, methanol and distilled water) of *A. aspera, C. serecia* and *M. pudica* depicted the presence of phenolics and tannins as proved by ferric chloride and lead acetate test. On the other hand, only ethanol and distilled water extracts of *A. aspera* and methanol extract of *M. pudica* showed positive results for phenolics and tannins using gelatin test. However, the leaf extracts of *C. serecia* depicted negative results for the presence of phenolics and tannins with gelatin test. Phenols possess a number of biological activities such as antioxidant, antiseptic, disinfectant fungicide and pesticides. The higher amount of phenols is important in the regulation of plant growth, development and disease resistance (Sharma *et al*., 2010). On the other hand, tannins bind to proline rich protein and interfere with protein synthesis (Yadav and Agarwala, 2011) as well as inhibit the pathogenic fungi (Pranoothi *et al*., 2014).

Meanwhile, all the leaf extracts of *A. aspera*; ethanol and methanol extracts of *M. pudica* gave positive results for saponins as proved by foam test. However, none of the extracts

Table 1: Phytochemical screening of leaf extracts

Sl. No.	Phytochemical tests	*Achyranthes aspera*			*Cassia serecia*			*Mimosa pudica*		
		E	M	Dw	E	M	Dw	E	M	Dw
I.	Alkaloids									
a.	Dragendorff's test	+ +	+ +	+	+	+	-	+ +	+ +	+
b.	Wagner's test	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+	+	+ +
II.	Flavonoids									
a.	Ammonia test	+ +	+ + +	+ + +	+ +	+ + +	+ +	+	+ +	+ + +
b.	Sodium hydroxide test	+	+	+	+	+	+ + +	+	+	+ +
III.	Phenolics and tannins									
a.	Ferric chloride test	+ + +	+ + +	+ + +	+ +	+ +	+ +	+ + +	+ + +	+ + +
b.	Gelatin test	+	-	+	-	-	-	-	+	-
c.	Lead acetate test	+ +	+ +	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +
IV.	Saponins									
a.	Foam test	+ + +	+ +	+	-	-	-	+	+ + +	-
V.	Terpenoids									
a.	Salkowski test	+ +	+ +	+ + +	+	+	-	+ + +	+ + +	+

E = Ethanol, M = Methanol, Dw = Distilled water, '+ + +' = Strongly present, '+ +' = Moderately present, '+' = Poorly present, '-' = Absent

Table 2: Total phenolic content (TPC) of plant sources

Sl. No.	Plant sources	Total phenolic content (mg/g dried leaf)		
		70% Ethanol	70% Methanol	Distilled water
1.	*Achyranthes aspera*	7.258 ± 1.785	13.985 ± 2.202	12.044 ± 1.603
2.	*Cassia serecia*	19.087 ± 1.911	16.932 ± 1.877	7.479 ± 1.777
3.	*Mimosa pudica*	27.496 ± 3.422	24.20 ± 1.465	9.324 ± 1.473

Mean ± Standard deviation

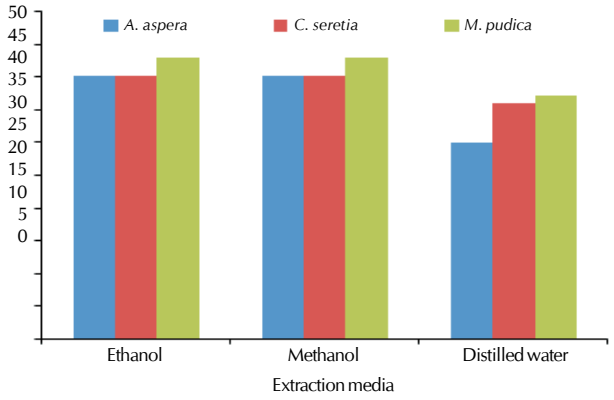

Figure 1: The yield of leaf extracts obtained using ethanol, methanol and distilled water

of *C. serecia* proved the presence of saponins using foam test. Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). They cause the leakage of proteins and degradation of cell wall enzymes from the cell (Pranoothi *et al*., 2014).

Further, all the extracts of *A. aspera* and *M. pudica* exhibited the presence of terpenoids using Salkowski test. Meanwhile, terpenoids were present in negligible amount in ethanol and methanol extracts of *C. serecia* leaves. Terpenoids are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleoresins.

Studies on phytochemical screening of weeds have been carried out by several authors and have reported for the presence of important phytochemicals in various extracts

of weed sources. In a similar study, weeds such as *Argemone maxicana*, *Cleome viscosa*, *Commelina benghalensis*, *Convolvulus arvensis*, *Crotalaria retusa* and *Crotalaria spesiosa* were found to contain alkaloids, saponins, tannins, steroids and flavonoids (Dhole *et al*., 2012).

Borkataky *et al*. (2013) reported that the methanolic extract of *A. conyzoides, E. odoratum and M. micrantha* contained alkaloids, saponins, flavonoids, phenolics and tannins, steroids and glycosides. *Euphorbia hirta*, *Evolvulus alsinoides*, *Commelina benghalensis*, *Croton bonplandianum* and *Achyranthes aspera* exhibited the presence of carbohydrates, flavonoids, lignin, terpenoids, steroids and phenols (Giri and Dhanalakshmi, 2015). Similarly, Licayan *et al*.,2015) found that *C. ternatea, M. penduala* and *P. lobata* leaf extracts contained alkaloids, carbohydrates, flavonoids, reducing sugars, tannins, saponins, and steroids.

Total phenolic content (TPC)

Total phenolic content of selected plant sources is described in Table 2. The results revealed that *A. aspera* leaves contained higher TPC in methanol (13.985 mg/g) extract followed by distilled water (12.044 mg/g) and ethanol (7.258 mg/g) extracts. On the other hand, ethanol (19.087 mg/g) extract of *C. serecia* yielded higher amount of TPC compared to methanol (16.932 mg/g) and distilled water (7.479 mg/g) extracts. Similarly, *M. pudica* also exhibited maximum TPC using ethanol (27.496 mg/g) against the phenolic concentration of methanol (24.20 mg/g) and distilled water (9.324 mg/g).

In a similar study, the total phenolic content was recorded maximum in the leaf of *Ocimum tenuiflorum* (Archana *et al*., 2011). Jyoti *et al*. (2016) also recorded higher total phenolic content in ethanol and methanol extracts of *E. globulus* and *C. citratus* respectively.

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