Preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb-an Ethnomedicinalplant.

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ABSTRACT:
Aim: The bioactive compounds present in the plant are responsible for the medicinal properties of the plant. The present investigation is aimed in screening the bioactive compounds present in the seed and fresh, dry leaves of *S.alata* - an important ethnomedicinal plant. The objectives of the work includes i) the screening of various bioactive compounds present in the seed, fresh and dry leaves of *S.alata*. ii) To identify the bio active compounds present in the fresh leaf extracts by Thin layer chromatography.
Methodology: Qualitative analysis for the presence of phytochemicals was performed using methanol, chloroform, petroleum ether and aqueous extracts of seed and both fresh and dry leaves of *Senna alata* by various standard techniques available [1-4]. Among the various extracts used for phytochemical analysis, only the fresh leaf extracts were proceeded for TLC for further identification of the phytoconstituents. The solvent system selected for the best results of TLC was ethyl acetate: methanol: water in the ratio 36:36:28.
Result: Phytochemical analysis revealed the presence of alkaloids, flavonoids, resins, anthraquinones, phlobatannins, tannins, saponins, glycosides and phenols in all the extracts in varying quantities. TLC analysis resulted in identification of 4 spots in benzene extract, 3 each for chloroform, methanol, aqueous extracts and 2 spots for the petroleum ether extracts.
Conclusion: Since the plant contains high amounts of these bioactive compounds, it is reliable to possess large number of medicinal values like anticancerous, antimutagenic, antioxidant, antifungal, laxative and antibacterial activities.
Keywords: Phytochemical studies, *Senna alata*, Thin Layer Chromatography.
Abbreviations: TLC: Thin Layer Chromatography.

INTRODUCTION

Senna alata L. Roxb. is an important ethnomedicinal plant known as *ringworm senna*, as the leaves of the plant are directly used for curing skin infections like ring worm. The plant is commonly known as *candle brush tree* or *empress candle*. The leaves of the plant are known to possess antimicrobial [5], anti-tumor [6,7], antioxidant [8], antimutagenic [9] and analgesic [10] activities. The leaves of the plant are also known to possess potent Antifungal properties [11, 12]. A 10 year study on human proved that the leaf extracts can be readily used as a herbal medicine for treatment of *Pityriasits versicolor*, a fungal infection without any side effects [13].The ointment made from the ethanolic extracts of the leaves is used as topical treatments on acute lesions of *dermatophilosis* in bovine and prevented its reoccurrence [14].

Apart from the above mentioned properties, the plant is known to possess hepatoprotective [15], antihyperglycemic[16] activities and is also used in the treatment of opportunistic infections in AIDS patients [17]. Recently, the extracts of the plant have been used in cosmetics and dermatological skin care products [18].

Herbal medicine has become more popular to cure many diseases due to its ease of availability, safety and less side effects. Phytochemicals are responsible for these medicinal properties of the plant [19, 20]. In view of its medicinal value, the present study is aimed to screen the pharmaceutically important bioactive substances from various extracts of seeds and leaves that contribute to the ethnomedicinal properties of *S.alata*.

MATERIALS AND METHODS

1. Preparation of Plant Material:

The healthy leaves and pods of *S.alata* were collected from the medicinal garden, Department of Biotechnology, Kakatiya University, Warangal. The leaves and pods were dried under shade and the seeds

were carefully separated from the pods. The completely dried leaves and seeds were ground into fine powder using an electric blender. This powder was used for the preparation of various extracts.

2. Preparation of Extracts:

2-3gms of each powder was taken into seperate 100 ml conical flasks and 50 ml of each solvent (Methanol, Water, Chloroform, Petroleum ether) were added separately. These flasks were labeled, plugged using cotton plugs and allowed to stand for 1-2hrs and filtered using Watmann No.1 filter paper. The filtrates obtained were used for the screening of secondary metabolites following standard procedures [1-4] (Kokate et al., 2009; Evans and Trease 2002; Khandelwal, 1995; *De* et al., 2010).

3. Phytochemical Tests:

I. Tests for Alkaloids:

A. **Mayer's test:** 1ml of each extract was mixed with a few drops of Mayer's reagent (Potassium Mercuric Iodide Solution). Formation of cream color precipitate indicates the presence of alkaloids.

B. **Wagner's test:** To 1ml of each extract was mixed with equal volumes of Wagner's reagent (Iodine in potassium iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

C. **Dragendorff's reagent test:** To 1ml of each extract, 2 ml of Dragendorff's reagent was added and mixed. To this 2 ml of dilute HCl was added. Formation of an orange colored precipitate indicates the presence of alkaloids.

D. **Hager's test:** To 2ml of each extract, a few drops of Hager's (Saturated picric acid solution) reagent were added. Formation of a bright yellow colored precipitate indicates the presence of alkaloids.

E. **Tannic acid test:** A pale yellow- brown colored precipitate obtained when the extracts are treated with 10% tannic acid conforms the presence of alkaloids.

F. **$FeCl_3$ test:** To 1-2 ml of all the extracts, add few drops of neutral ferric chloride solution. Deposition of yellow precipitate indicates the presence of alkaloids.

II. Tests for glycosides:

A. **Raymond's test:** The extracts when treated with dinitrobenzene in hot alkali, pink to violet color will be observed indicating the presence of glycosides.

B. **Legal's Test:** The extracts when treated with pyridine and alkaline sodium nitroprusside solution was added, appearance of cherry red color indicates the presence of glycosides.

C. **Bromine Water test:** The extracts when treated with bromine water yielded a pale yellow color indicates the presence of glycosides.

D. **Keller Killiani test:** 1ml of the extracts were dissolved in 1ml of glacial acetic acid and cooled, after cooling, 2-3 drops of ferric chloride was added. To this solution 2ml of conc. H_2SO_4 was added carefully along the walls of the test tube. Appearance of reddish brown colored ring at the junction of two layers indicates the presence of glycosides.

E. **Conc. H_2SO_4 test:** To 1ml of the extracts, 1ml of conc. H_2SO_4 was added and allowed to stand for 2 min. a reddish color precipitate indicates the presence of glycosides.

F. **Molish's test:** 2-3 drops of molisch reagent was added to the extracts and mixed well. To this, a few drops of conc. H_2SO_4 was added carefully. Formation of reddish-purple colored ring at the junction of two layers indicates the presence of glycosides.

III. Test for anthraquinones: 1-2ml of the extracts were mixed with equal volumes of benzene and then about 1ml of 10% ammonia solution was added. Formation of red color on addition of ammonia indicates the presence of anthraquinones.

IV. Test for Phlobatannins: 10ml of the extract was mixed with 2-3ml of 10% HCl in a boiling tube and the contents were boiled for 5-6 min. Formation of red colored precipitate indicates the presence of phlobatannins.

V. Test for Resins: To the extracts, 3-4 ml of CuSO4 solution was added separately and the tubes were shaken vigorously for 1-2 min. the resulting solution was allowed to separate. Formation of green colored precipitate indicates the presence of resins.

VI. Test for Quinones: The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

VII. Test for Saponins: 5ml of each extract is taken in a test tube and shaken vigorously to obtain a stable froth. To this frothing solution, 5-6 drops of olive oil was added. Formation of an emulsion indicates the presence of saponins.

VIII. Tests for Phenols:

A. **Ellagic acid test:** The extracts were treated with few drops of 5% (w/v) glacial acetic acid followed by 5% (w/v) NaNO2 solution. Formation of muddy brown color indicates the presence of phenols.

B. **Phenol test:** 2ml of the extracts were separately treated with 1ml of $FeCl_3$ solution. Development of an intense color conforms the presence of phenols.

IX. Tests for Tannins:

A. **Ferric chloride test:** The extracts were treated separately with few drops of FeCl3 solution. Formation of blackish precipitate indicates the presence of tannins.

B. **Gelatin test:** The extracts were treated with few drops of gelatin solution. Formation of white precipitate indicates the presence of tannins.

C. **Lead acetate test:** To 1-2 ml of extracts, basic lead acetate was added separately. Formation of bulky red precipitate indicates the presence of tannins.

D. **Alkaline Reagent test:** 1-2 ml of extracts were treated with a solution of sodium hydroxide. Appearance of yellow to red color indicates the presence of tannins.

E. **Mitchell's test:** The extracts when treated with a solution of iron and sodium tartarate gives a water soluble, ammonium citrate insoluble complex indicating the presence of tannins.

X. Tests for Flavonoids:

A. **Zinc-HCl reduction test:** To all the extracts add a pinch of Zinc dust and a few drops of Conc. HCl. Formation of deep red color indicates the presence of Flavonoids.

B. **Lead-acetate test:** To 1-2 ml of all the extracts, add few drops basic lead acetate solution. Formation of reddish brown precipitate indicates the presence of flavonoids.

C. **Shinoda's test:** To 1-2 ml of all the extracts, add a small piece of magnesium paper and add a few drops of conc. HCl carefully along the walls of the tube. Appearance of red color indicates the presence of flavonoids.

D. **FeCl3 test:** To 1-2 ml of all the extracts, add few drops of neutral ferric chloride solution. Deposition of blackish red precipitate indicates the presence of flavonoids.

E. **Alkaline Reagent test:** to 1-2 ml of all the extracts, a solution of sodium hydroxide is added. Appearance of yellow to red color indicates the presence of flavonoids.

XI. Test for Coumarins: 1-2 ml of all the extracts were taken in separate tubes and covered with a piece of paper soaked in NaOH and heated. When these tubes yield a yellow fluorescence under UV light indicates the presence of coumarins.

XII. Test for Sterols:

A. **Liebermann-Burchard test:** To 1-2 ml of all the extracts, a few drops of acetic anhydride solution was added. To this mixture, a few drops of Conc. H_2SO_4 was added carefully along the walls of the test tube. Formation of reddish brown ring at the junction of two layers indicates the presence of steroids.

B. **Salkowski test:** to 1-2 ml of all the extracts, 5ml of chloroform was added. To the above mixture, 1ml of conc. H_2SO_4 was added carefully along the walls of the tube and mixed. The formation of reddish color in the lower layer indicates the presence of steroids.

XIII. Test for Terpenoids: To 1-2 ml of all the extracts 1% HCl was added and allowed to stand for 5-6 hours. Later, these extracts were treated with 1ml of Trim-Hill reagent (a solution of **10** ml of acetic acid, 1 ml of 0.2% copper sulphate in water and 0.5 ml of concentrated hydrochloric acid) and heated in a boiling water bath for 5-10 minutes. Formation of bluish green color indicates the presence of terpenoids.

Thin layer chromatography

Thin Layer Chromatography (TLC) is a technique used for the separation of a mixture of compounds. TLC analysis of fresh leaf samples was performed using commercially available aluminium sheets of silica gel 60 F_{254} (Merck). All the fresh leaf extracts were prepared in their respective solvents just before application onto the silica gel plate and spotted as a single spot using seperate capillary tubes [21].Different solvent systems were tried to select a suitable solvent system. The TLC plates were kept in the chamber containing the solvent system (mobile phase) taking care that the mobile phase is not coming in contact with the spots at the bottom of the TLC plate. The TLC chamber is covered at the top and the plate is allowed to develop. After the plate has developed, the solvent front was marked and the plate is allowed to dry. The separated components were detected using appropriate visualizing agent like iodine vapors. The qualitative analysis of the separated components can be carried out by calculating the R_f values.

RESULTS

The results on preliminary phytochemical screening of seed, fresh and shade dried leaves of *S.alata* are shown in Tables 1- 2. The phytochemical analysis of seed extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, resin and glycosides in all the extracts except methanolic extracts.

Sterols were present only in the chloroform extract and phenols only in the aqueous extracts. Lignins and quinones are completely absent in all the seed extracts (Table 1).

Phytochemical screening of fresh and dry leaves showed the presence of alkaloids, flavonoids, anthraquinones, saponins, glycosides and tannins in high amounts in all the extracts except methanolic extract. Resins and phenols are present in moderate amounts. Sterols are present only in chloroform extracts. Terpenoids and phlobatannins are present only in fresh leaf extracts and quinones only in benzene and aqueous extracts of dry leaf (Table 2).

The solvent system used for TLC was ethyl acetate: methanol: water in the ratio 36:36: 28. TLC analysis of fresh leaf extracts resulted in the identification of 4 spots with the R_f values of 0.069, 0.21, 0.45 and 0.90 in benzene, 0.24, 0.57, 0.63 in chloroform, 0.24, 0.57, 0.62 in methanol 0.18, 0.54 in petroleum ether and 0.15, 0.454, 0.54 in the aqueous extracts of *S.alata* (Fig.1).

DISCUSSION

The results of the present study indicates the presence of glycosides, alkaloids, resins, tannins and anthraquinones in all the seed extracts except in the methanol extract (Table-1) and analysis of fresh and dry leaf extracts of *S.alata* indicates the presence of alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids, anthraquinones, resins and steroids in the fresh leaf extracts except in the methanolic extract and glycosides, alkaloids, saponins, tannins, flavonoids and anthraquinones in dry leaf extracts except in the methanol extract (Table-2). These results support the work of Christy Jeyaseelan *et al*., [22]. They reported that the fresh leaf aqueous extracts of *S.alata* showed the presence of glycosides, alkaloids, saponins, tannins, flavonoids, terpenoids and anthraquinones. The difference in the above results in the respective solvents may be due to the interaction of the phytoconstituents with the solvent system or the process employed for extraction.

Anthraquinones and alkaloids are present in all the extracts (seed, fresh and dry leaf extracts). Anthraquinones are aromatic organic compounds, yellow coloured crystalline compounds, poorly soluble in water. They have a wide range of application in the fields of agriculture, medicine and paper making industry (www. Schri.com). Anthraquinones are used as laxatives in medicine antimalarials and antineoplastics (in chemotherapy) in the treatment of cancers. The laxative and anti tumor effects of the plant are due to the presence of anthraquinones.

Alkaloids are a group of naturally occurring chemical compounds and chief class of plant secondary metabolites. They are bitter to taste and are toxic to other organisms [23] and hence act in inhibiting microbial growth. The antibacterial and antifungal properties of the plant may be due to the presence of alkaloids.

Flavonoids are present in fresh and dry leaf extracts of the plant but were absent in the seed extracts. They are a group of polyphenolic compounds that have potent antimicrobial [24], anti inflammatory [25, 26] actions. Flavonoids are free radical scavengers which prevent oxidative cell damage and have strong anti-cancer activity [27]. The antioxidant, anti-inflammatory, antimutagenic and antimicrobial activities of the plant may be due to the presence of flavonoids.

Glycosides are present in seed, both fresh and dry leaf extracts of the plant. Glycosides are the molecules in which a sugar is bound to a non sugar moiety usually a small organic molecule by a glycosidic bond. A number of glycosides are used for storage purpose. A class of glycosides known as cardiac glycosides have an important role in medicine because of their action on heart and used in cardiac insufficiency [28].

Phenols are one of the largest and most ubiquitous group of plant metabolites. A number of biological properties such as antiapoptosis, antiageing, anticarcinogen, anti-inflammation and cell proliferating activities are attributed with phenolics [29]. The anti-inflammatory, anti- apoptotic and anti-ageing properties of the plant may be due to the presence of phenols.

Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane due to their physiological activities such as anti-oxidant, antimicrobial and anti-inflammatory properties. The use of *S.alata* leaves directly for healing fungal infections has long been in practice. This property may be due to the presence of tannins. Tannins have been used since past as tanning agents as they posses astringent, anti inflammatory, anti diarrheal, antioxidant and antimicrobial activities [30]. Saponins have traditionally used in detergents, pesticides and molluscicides in addition to their industrial applications such as foaming and surface active agents. They help in controlling cardiovascular diseases and in controlling cholesterol in humans [31]. In addition to their use in industry, saponins also have a wide range of medicinal applications [32].

TLC analysis resulted in the formation of 4 spots in benzene, 3 each in aqueous, methanol and chloroform extracts and 2 in the petroleum ether extracts (Fig.1) which indicates the presence of varying quantities of phytochemicals in the respective extracts.

CONCLUSION

Qualitative analysis of seed, fresh and dry leaf extracts of *S.alata* showed the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones, resin and glycosides in all the extracts except methanolic extracts.

Sterols were present only in the chloroform extracts and phenols only in the aqueous extracts. Lignins and quinones are completely absent in all the seed extracts. Fresh and dry leaf extracts showed the presence of alkaloids, flavonoids, anthraquinones, saponins, glycosides and tannins in high amounts in all the extracts except methanolic extract. Resins and phenols in moderate amounts. Sterols are present only in chloroform extracts. Terpenoids and phlobatannins are present only in fresh leaf extracts and quinones only in benzene and aqueous extracts of dry leaf. The choice of the solvent system and the procedure for extraction may affect the quality and quantity of phytochemicals in the leaf and seed extracts.

TLC analysis revealed the presence of different types of phytochemicals based on the number of spots. The compounds present in the mixture are to be screened and purified for qualitative estimation.

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Table-1: Phytochemical analysis of seed extracts of *S. alata*.

Phytochemical test		Methanol Extract	Petroleum ether Extract	Benzene Extract	Chloroform Extract	Aqueous Extract
ALKALOIDS	Dragendorff's test	-	++	++	++	++
	Mayer's test	-	++	++	++	++
	Wagner's test	-	++	++	++	++
	Hager's test	-	++	++	++	++
	Tanicacid test	-	++	++	++	++
GLYCOSIDES	Raymond's test	-	++	++	++	++
	Legal's test	-	++	++	++	++
	Bromine water test	-	++	++	++	++
	Kellar Kiliani test	-	++	++	++	++
	Conc. H_2SO_4 test	-	++	++	++	++
	Molisch test	-	++	++	++	++
TANNINS	$FeCl_3$ test	-	++	+	++	++
	Gelatin test	-	++	+	++	++
	Lead acetate test	-	++	-	++	++
	Alkaline reagent test	-	++	+	++	++
	Mitchell's test	-	++	+	++	++
	Zn-Hcl Reduction test	-	++	++	++	++
ANTHRA QUINONES		-	++	++	++	++
FLAVONOIDS	Lead acetate test	-	++	++	++	++
	$FeCl_3$ test	-	++	++	++	++
	Shinoda's test	-	++	++	++	++
	Alkaline reagent test	-	++	++	++	++
STEROLS	Libermann Burchard test	-	-	-	++	-
	Salkowski test	-	+	-	++	-
COUMARINS		–	–	–	–	–
RESINS		-	++	++	++	++
PHENOLS	$FeCl_3$ test	-	-	-	-	+
	Elagic acid test	-	-	-	-	+
Terpenoids		-	-	-	-	-
PHLOBA TANNINS		-	-	-	-	-
QUINONES	Alcoholic KOH test	-	-	-	-	-
SAPONINS	Foam test	+	+	+	+	+

++= strongly present, += present, -= absent.

Table-2: Phytochemical analysis of leaf extracts of *S. alata*

Phytochemical test		Methanol Extract		Petroleum ether Extract		Benzene Extract		Chloroform Extract		Aqueous Extract	
		F	D	F	D	F	D	F	D	F	D
ALKALOIDS	Dragendorff's test	-	-	++	++	++	++	++	+	++	++
	Mayer's test	-	-	++	-	++	-	++	-	++	++
	Wagner's test	-	-	++	++	++	++	++	++	++	++
	Hager's test	-	-	++	++	++	++	++	++	++	++
	Tanicacid test	-	-	++	++	++	++	++	++	++	++
GLYCOSIDES	Raymond's test	-	-	++	++	++	++	++	-	++	++
	Legal's test	++	-	++	++	++	++	++	++	++	++
	Bromine water test	-	-	++	-	++	-	++	-	++	++
	Kellar Kiliani test	-	-	++	++	++	++	++	++	++	++
	Conc. H_2SO_4 test	++	-	++	++	++	++	++	++	++	++
	Molisch test	-	-	++	-	++	++	++	++	++	++
TANNINS	$FeCl_3$ test	+	-	++	++	++	+	++	+	++	+
	Gelatin test	-	-	++	++	++	+	++	+	++	+
	Lead acetate test	-	-	++	++	++	-	++	+	++	+
	Mitchell's test	-	-	++	++	++	-	++	-	++	-
ANTHRA-QUINONES		++		++	++	++	++	++	++	++	++
FLAVONOIDS	Zn-HCl reduction test	-	-	++	++	++	++	++	++	++	++
	Lead acetate test	+	-	++	++	++	++	++	++	++	++
	$FeCl_3$ test	-	-	++	++	++	++	++	++	++	++
	Shinoda's test	+	-	++	++	++	++	++	++	++	++
	Alkaline reagent test	-	-	++	++	++	++	++	++	++	++
STEROLS	Libermann Burchard test	-	–	–	–	–	–	++	++	-	-
	Salkowski test	–	–	–	–	–	–	++	++	-	-
COUMARINS		–	–	–	–	–	–	–	–	--	--
RESINS		-	-	++	-	++	++	++	-	++	++
PHENOLS	$FeCl_3$ test	-	-	++	-	++	++	++	++	++	++
	Elagic acid test	+	-	-	-	+	+	+	+	++	++
TERPENOIDS	Trim-Hill test	–	–	++	–	++	-	++	-	++	-
PHLOBA-TANNINS		+	-	+	+	+	-	+	-	+	+
QUINONES	Alcoholic KOH test	-	-	-	-	-	+	-	-	-	+
SAPONINS	Foam test	++	-	++	++	++	++	++	++	++	++

F= Fresh leaf extract; D= Dry leaf extract;
++= strongly present, += present, -= absent

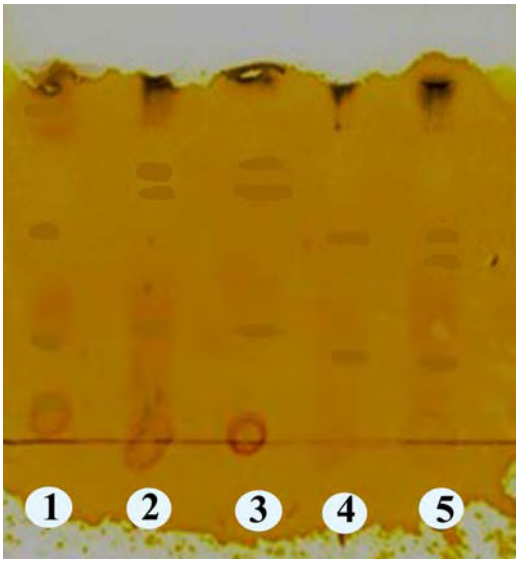

Fig-1: TLC analysis of fresh leaf extracts of *S.alata*

1. Benzene extract 2. Chloroform extract 3.Methanol extract 4. Petroleum ether extract 5. Aqueous extract