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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF SENNA *UNIFLORA* (MILL.) H.S. IRWIN & BARNEBY

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ABSTRACT

The traditional medicines involve the use of plant extracts particularly the bioactive components present in it. This type of study provides the health application at affordable cost. Secondary metabolites in plants are responsible for various medicinal uses of plants. Hence in the present study phytochemical screening of Senna *uniflora* (Mill.) H.S. Irwin &Barneby was carried out. Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals like Alkaloids, Flavonoids, Quinones, Saponins, Sterols, Terpenoids, Tri terpenoids and etc., of Ethanol, Methanol, Acetone, Hexane and Water extracts by Maceration method. The Antimicrobial activity of the above five extracts was also studied for six different microorganisms by zone inhibition method. Methanol and Hexane extracts have good zone inhibition ranges against microorganisms. Every parameter is compared with a standard drug. The results suggest that this plant extracts possess potential antimicrobials and lead to the isolation of novel compounds.

KEYWORDS: Senna *uniflora*, Maceration, phytochemical screening, bioactive components, antimicrobial activities.

1. INTRODUCTION

Plants are rich source of secondary metabolites with interesting biological activities. Many of the phytochemicals are found to alter numerous cell functions[1]. Plants have been used as medicine for thousands of years and also a hallmark in the search of new medicine[2]. Many plant species have been used in traditional medicine to treat many health problems. Even today compounds from plants continue to play a major role in primary health care as

therapeutic remedies in many developing countries[5]. The application of modern instruments and techniques for standardization and formulation is the need of the hour. A lot of physico and chemico data are available. But there are no advanced and modern methods to describe, the identification and quantification of active constituents in the plant materials. In some of the works, the researchers describe a phytochemical screening method to detect some compounds like sweet steviol(13-hydroxy-ent-kaurenoic acid) glycoside constituents. Such a study was initiated to locate new sources of sweet ent- kaurene glycosides within the genus Stevia[3].

The synonyms of taxon Senna *uniflora* (Mill.) H.S. Irwin &Barneby are Cassia *sericea* SW. and Cassia *Uniflora* Mill.(basionym)[17]. The plant belongs to Fabaceae (alt. Leguminosae) subfamily : Caesalpinioideae tribe : Cassieaesubtribe: Cassiinae and also placed in Caesalpiniaceae[4,17]. The common name is one leaf senna. Decoction of mature leaves laxative, useful in curing ring-worm and skin diseases[5]. It was reported for the first time from Eastern Karnataka and subsequently from Pune, Madhya Pradesh (Dhar and Thabua district). Andrapradhesh, Kerala, Mangrol, Laxmipura villages, 24 km away from South East of Chittorgarh, Rajasthan[6], and Tamilnadu[7]. It is distributed in Brazil, West Indies, Central America and Mexico, introduced in many tropical countries as a weed. In India, this taxon is found in Maharastra and Karnataka. It is reported as new records for Chakarnagar area along the Chambal ravine of Etawah district [11]. The poultice of leaves is applied to wounds and the extract of leaves is reported to cure eczema. The roots are used to combat dropsy. The plant has also been reported to smother the growth of Parthenium hysterophorus L. in many states in India[13], Dharwar and Belgaum[1] and Andhra Pradesh[16]. It has allelopathic potential of this plant was valued in a Green house experiment with dry Ipomoea tricolour mixed with sterile and non-sterile soil in pots[10]. Bioactive compound Tricolorin A exhibited no inhibitory effect on Senna *uniflora* [10]. The aqueous solution of Senna *uniflora* (Mill.)is useful in the removal of copper (II) ions from industrial effluents by adsorption method[12,14]. The benzene and alcohol extracts of Senna *uniflora* showed significant inhibition on paw oedema albino rats [15].

The medicinal prominence of the plant is taken into consideration and, the ethanol extract of the whole plant of Senna *uniflora* was analyzed for the first time in GC-MS[8].

2. MATERIALS AND METHODS

2.1 Plant Materials and Preparation of Plant Extracts

The plant material was extracted by cold maceration for 72 hours with various solvents with intermittent agitation. After incubation, the extracts were filtered through Whatman filter paper and the extracts were collected and stored at 4°C in refrigerator till further use.

A Voucher Specimen was deposited with the Botanical Survey of India (BSI), Coimbatore, TN, South India under the accession number BSI/SRC/5/23/2012-13 Tech. 956 (11/09/2012).

Fig.1. Senna*uni flora* plant

2.2 Phytochemical screening

The different qualitative chemical tests were carried out on the aqueous extract using standard procedures to identify the constituents as described by Sofawara (1993), Trease and Evans (1989), Harborne (1973) and Edeoga (2005) [5]. Phytochemical examinations were carried out for all the extracts as per the standard methods [5] , [9].

1. Detection of alkaloids

Extracts dissolved individually in dilute Hydrochloric acid and filtered [19].

a. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2. Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates[9].
b. Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars[9].

3. Detection of Cardiac glycosides

a. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides[9].

4. Detection of saponins

a. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins[9].
b. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins[9].

5. Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of sterols[9].

a) LibermannBurchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols[9].

6. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols[9].

7. Detection of tannins

a) Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins [9].
b) 1ml of filtrate with 2ml of Ferric chloride gives Dark green colour indicates the presence of tanins.

8. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with aa few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids[9].

9. Detection of proteins and aminoacids

a) Xanthoproteic Test: The extracts were treated with a few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins [9].

b) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acid [9].

10. Detection of Anthocyanins

Add 1ml of filtrate with 5ml of dilute HCl, the appearance of pale pink colour indicates the presence of the Anthocyanins[5].

11. Detection of Steroids

To 1ml of the filtrate add 10ml chloroform and 10ml of H_2SO_4 slowly by the sides of the test tube. Upper layer turns red and sulphuric acid layer shows yellow with green fluorescence [5].

12. Detection of Terpenoids

Take 1ml of the filtrate with 2ml $CHCl_3$ and carefully add a few drops of conc H_2SO_4. An interface with a reddish brown colouration is formed[5].

13. Detection of Quinone

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow precipitate (or colouration)[21].

14. Detection of Coumarins

a) Evaporate 5 ml of ethanolic solution, dissolve the residue in 1-2 ml of hot distilled water and divide the volume into two parts. Take half the volume as a witness and to add another volume of 0.5 ml 10% NH_4OH. Put two spots on filter paper and examined under UV light. Intense fluorescence indicates the presence of coumarins[23].

b) 0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins[24].

15. Detection of Triterpenoids

Salkowski test: A few drops of concentrated sulphuric acid was added to the chloroform solution, shaken and allowed to stand, appearance of golden yellow color indicates the presence of triterpenes[22].

16. Detection of Lignins

Labat test: To the extract added gallic acid, it develops olive green color indicating the positive reaction for lignins[22].

2.3 Antibacterial agents and their importance

An antimicrobial agent is a compound that selectively abolishes or prevents the growth of micro-organisms. Antibiotics, disinfectants and antiseptics are all categorised as antimicrobial agents. Based on their action, they can be broadly classified into two, the effect of an antimicrobial is whether it terminates (bactericidal) or it inhibits the growth of the micro-organism (bacteriostatic). These effects are not reciprocally exclusive; antimicrobials can kills as well as make static. An antimicrobial agent's range of activity details its effect on various micro-organisms. The main micro-organisms are usually bacteria (sometimes including bacterial spores), viruses and fungi. The antiseptic qualities of aromatic and medicinal plants and their extracts have been acknowledged since ancient times, while efforts to illustrate these properties in the laboratory date back to the early 1900s.The antimicrobial properties of plant volatile oils and their components from a wide spectrum of plants have been evaluated and swotted. It is clear from these studies that these plant secondary metabolites have potential in medical techniques and applications in the cosmetic, food and pharmaceutical industries[18]. The anti-microbial activity for the given sample was carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996, Vol II A-105). The test microorganisms of Staphylococcusaureus, Basillussubtilis, E.Coli, Klebsiellaaerogenes, Aspergillusniger, Candida albicans were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose agar medium for bacteria and Fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5µg/disc for bacteria; Nystatin 100 units / disc for fungi).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The results of the phytochemical screening are given in the Table 1. It reveals the presence of various phytochemicals like Alkaloids, Flavonoids, Quinones, Saponins, Sterols, Terpenoids, Tri terpenoids of Ethanol, Methanol, Acetone, Hexane and Water extracts by Maceration method. Phytocompounds Anthocyanin, Glycosides, Cardiac glycosides are absent in all the five extracts.

Table No.1- Phytochemical Screening of *Sennauniflora* whole plant

S. No.	PHYTOCHEMICAL SCREENING			SOLVENTS				
	Phyto components	**Name of the test**	**Observation**	**EtOH**	**MeOH**	**Acetone**	**Hexane**	**Water**
1	Alkaloids	Wagners Test	Reddish brown precipitate	+++	+++	+++	++	+++
2	Coumarins		Yellow	-	-	+	-	+
3	Anthocyanin Test		Pink	-	-	-	-	-
4	Amino Acid / Protein Test	Biuret Test	Violet	-	-	-	-	-
		Ninhydrin Test	Purple	-	-	-	-	+
		Xanthoprotein Test	Yellow	+	+	+	+	+
5	Glycosides	Kellar-Kiliani	Reddish brown	-	-	-	-	-
6	Cardiac glycosides Test	Neutral $FeCl_3$ Test	Blue	-	-	-	-	-
		Legal's Test	Blood red	-	-	-	-	-
7	Carbo-hydrates	Molisch's Test	Reddish Violet ring	-	-	-	-	+
		Benedicts Test	Orange red	-	-	-	-	-
8	Flavonoids	Neutral $FeCl_3$	Blackish green	-	-	-	-	+
		Zinc-HCl reduction	Magenta	-	+	-	-	-
		Alkaline Reagent Test	Yellow	+	+	+	+	-
9	Coumarins		Yellow decolorises while adding **Conc**.HCl	-	-	+	-	+
10	Quinone		Greenish yellow	+	+	+	+	+
11	Phenolic		Intense Colour	+	-	-	-	-
12	Saponins	Froth Test	1cm layer of foam	+	+	+	-	-
13	Sterols	Salkowski Test	Golden yellow in lower layer	+	+	-	+	-
		Libermann-Buchard Test	Reddish brown ring	++	+	+	-	-

14	Terpenoids		Reddish brown	+	+++	+	-	+
15	Triterpenoids	Salkowski Test	Golden yellow	+	+	++	+	+
16	Lignin	Labat Test	Olive green	+	+	+	-	-

3.2 Antimicrobial Activity

The results of antimicrobial activity are given in the Table 2 and in figure 2 it is explained using a bar chart.The Antimicrobial activity of the above five extracts was also studied for six different microorganisms by zone inhibition method. All the five extracts showed concentration dependent activity against all the tested bacteria and fungi. The zone inhibition recordranged from 10 to 20 mm at various extracts. Methanol extract exhibited maximum zone inhibition against Gram positive bacteria Staphylococcus aureus (20 mm), Gram negative bacteria E.Coli (20 mm), and the fungal pathogen Candida albicans (20 mm). Hexane extract showed good zone inhibition, ranges from 12 to 20 mm . It showed maximum zone inhibition against the fungal pathogen Candida albicans (20 mm). Water extract that is aqueous extract showed zone inhibition ranges from 14 to 20 mm. It displayed maximum zone inhibition against Gram positive bacteria Staphylococcus aureus (20 mm). Every parameter is compared with standard drugs i.e., Ciprofloxacin 5µg/disc for bacteria; Nystatin 100 units / disc for fungi. The solvent used is DMSO.The results suggest that these extracts of the *senna*uniflorapossess potential antimicrobials and leads to the isolation of novel phytoconstituentsin it.

Table No.2- Antimicrobial activity of *Sennauniflora* whole plan

Standard - Ciprofloxacin 5µg/disc for bacteria; Nystatin 100 units / disc for fungi.

Solvent – DMSO

S.No	Name of the Microorganisms	Zone Inhibition					
		EtOH Ext	MeOH Ext	Acetone Ext	Hexane Ext	Water Ext	Standard Drug
1	Staphylococcus aureus (NCIM 2079)	16	18	17	16	20	35
2	Basillussubtilis (NCIM 2063)	14	14	16	16	10	40
3	E.Coli (NCIM 2065)	17	20	18	12	14	38
4	Klebsiellaaerogenes (NCIM 2098)	12	10	12	16	18	30
5	Aspergillusniger (NCIM 105)	14	14	16	17	14	35
6	Candida albicans (NCIM 3102)	18	20	14	20	16	32

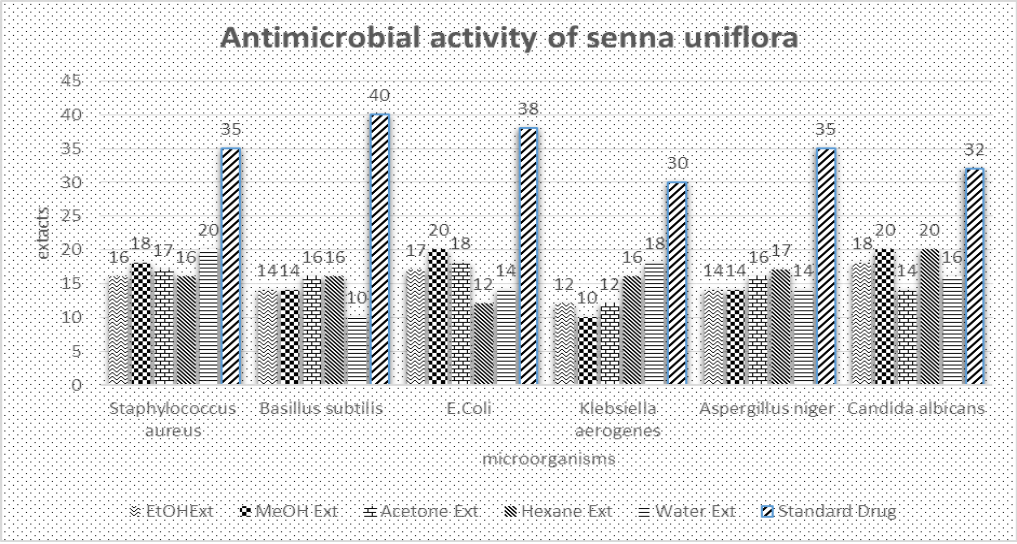

Fig.2. Antimicrobial activity Senna *uniflora* Zone inhibition method

The anti-microbial activity for the given sample was carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996, Vol II A-105). The test microorganisms of Staphylococcusaureus, Basillussubtilis, E.Coli, Klebsiellaaerogenes, Aspergillusniger, Candida albicans were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose agar medium for bacteria and Fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5µg/disc for bacteria; Nystatin 100 units / disc for fungi).

4. CONCLUSION

The medicinal plants are rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. *Senna*uniflora has significant anti-microbial activity can be attributed to the presence of alkaloids, flavonoids, quinones, saponins, sterols, terpenoids, tri terpenoids and etc., Exploration of these pharmacological properties involves further investigation of these dynamic ingredients by implementation techniques of extraction, purification, separation, crystallization and identification of various bioactive compounds for the therapeutic interest to cure various human ailments.

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