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PHYTOCHEMICAL ANALYSIS ON ETHANOLIC EXTRACT OF BERRIES OF *SOLANUM TORVUM* SWARTZ

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ABSTRACT

Solanum torvum swartz , a solanaceae family plant is used by traditional system of medicine. This study aimed to determine the phytochemical constituents present in various extracts of solanum torvum. viz , aqueous, ethanolic, methanolic extracts of solanum torvum. The phytochemical parameters such as ash value, extractive value and total fibre content were estimated. The qualitative analysis of each extract showed the presence of reducing sugar, saponins, alkaloids, phenols, flavonoids except anthraquinones. Quantitative determination of total phenols and flavonoids in extract of solanum torvum showed 16.4mg GAE/g extract of solanum torvum and 2.8mg QE/q solanum torvum respectively. Phytochemicals are frequently used in chemotherapeutic treatment (or) may be used as chemoprotective agents with chemoprevention. Solanum torvum which is a multipurpose constant slender herb. It contains number of potentially active constituents such as phenols and flavonoids act as anti oxidant, anti inflammatory, lowering blood pressure and cholesterol, anti microbial activity. Total phenolic and flavonoid content were evaluated using the Folin- Ciocalteus method and AlCl3 test respectively. The study showed that extracts contains diversity of phytochemicals in appreciable in amount that can expertly keep the body against oxidative stress triggered by free radicals and therefore be used as source of potent natural products. .

Key words: Solanum torvum , HPLC, Fenton's method and Free Radicals..

INTRODUCTION

Plants have been used in traditional medicine for several thousands years. The knowledge of medicinal plants had been accumulated in the course of many centuries based on different medicinal system such as Ayurveda, Unani and Siddha. In India, it is reported as traditional healers use 2500 plant species and 100 species of plant serve as regular source of medicine. During the last few decades there had been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. Plants have an almost limitless ability to synthesize aromatic. Substances mainly secondary metabolites of which atleast 12,000.Have been isolated a number estimated to be less than 10% of the total. In many cases these substances serve as the molecules of plant defense against predation by microorganisms, insects and herbivores. Further, some the phytochemical which may involve in plant odour (terpenoids),pigmentation(tannins and quinines) and flavors(capsacin).Some of medicinal plants used in Ayurveda, Unani, Siddha and in folk medicine for treating several ailments Including microbial infections, diarrhea and diabetes [1-4].

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BOTANICAL CLASSIFICATION			
Scientific classification:		**Vernacular names:**	
Kingdom	**Plantae**	**Region/Language**	**Vernacular name**
Devision	Magnoliophyta	Sanskrit	Brihati
Class	Magnoliopsida	Marathi	Marang
Order	Solanales	Hindi	Bhurat, Bhankatiya
Family	Solanaceae	English	West Indian Turkey Berry
Genus	Solanum L.	Tamil	Sundaikkai
Species	Solanum torvum swartz	Folk	Ran-Baingan, goth- begun

BOTANICAL DESCRIPTION:

LEAVES:

Leaves simple, alternate, broadly ovate elliptic, variable in size, 10-15cm long, 8-10cm wide , margins with broad lobes, deeply cut in juvenile phases, shallow in mature leaves, apex acute to obtuse, base somewhat sagittate to auriculate, equal or Oblique, petioles 2-5cm long.

FLOWERS:

The small, white flowers occur in large clusters, with simple, mostly glandular hairs on axes; corolla bright white, to 2.5cm across, lobed about 1/3 of its length; lobes not recurved; Stamens with prominent anthers.

FRUITS:

Fruits are berries that are yellow when fully ripe. They are thin-fleshed and contain numerous flat, round, brown seeds.

ODOUR: Pepper-like

SEEDS:

Seeds numerous, drab brownish, flattened, discoid, 1.5-2mm long slightly reticulate, self-compatible.

TASTE: Bitter and acrid

PARTS USED: Plant, leaves, fruits and root.

FAMILY : Solanaceae

MATERIALS AND METHODS

Extract preparation:

The health plant berries of solanum torvum was collected from western ghats of madurai range,Tamil nadu,India.The were collected in early morning and were washed in tap water and shade dried for 10 days.The shade dried plant material was powdered using mixer grinder and that powder was subjected to Soxhlet extraction with petroleum ether and methanol(60^0c) for 24 hrs.Each solvent extract was distilled and condensed at 40^0c.The condensed extract was stored at room temperature in air tight bottles and used for further studies.

PHYTOCHEMICAL STUDIES:

The presence of bioactive phytocompounds was secondary metabolites from the leaves of solanum torvum and solanum nigram were qualitatively analysed by thin layer chromatography. Solid phase of silica gel were kept in hot air oven in 100^0c for 20 minutes. Silica powder was mixed with petroleum ether and makes slurry. 20×20 cm TLC glass plates covered with that slurry and allowed to air dried. After drying the plates were kept in hot air oven in 72^0c for 1 hr. After developing the plates the condensed filtrate was spotted using capillary tube. The different spots were seperated using a different solvent mixture act as mobile phase [5-9].

TLC study of alkaloids:

The powdered seeds of solanum torvum was wetted with a half diluted NH_4OH and lixiviated with Et OAc for 24hrs at RT. The organic phase is separated from the acidified filtrate and basified with NH_4OH(p^H 11-12). It is extracted with chloroform (3x), condensed by evaporation and used for chromatography. The alkaloids spots were separated using the solvents mixture chloroform and methanol(15:1). The colour and hR value of the separated alkaloids were recorded both under Ultra Violet(UV254nm) and visible light after spraying with Dragendorff's reagent.

TLC of flavonoids:

One gram powdered seeds of solanum torvum was extracted with 10ml methanol on water bath(60^0c/5min). The filtrate was condensed by evaporation, added a mixture of water and EtOAc(10:1ml), and mixed thoroughly. The EtOAc phase thus retained is used for chromatography. The flavonoid spots were saperated using chloroform and methanol(19:1) solvent mixture. The colour and hRf value of these spots were recorded under ultraviolet($UV_{254\ nm}$) light.

TLC study of glycosides:

The powdered seeds of solanum torvum was extracted with 70% EtOH on rotary shaker (180 thaws/min) for 10hr.70%lead acetate is added to the filtrate and centrifuged at 5000rpm/10min.The

supernatant was further centrifuged by adding 6.3%Na2 Co_3 at 10000rpm/10min.The retained supernatant was dried, redissolved in chloroform and used for chromatography. The glycosides were separated using EtOAC –MeOH- H_2O(80:10:10)solvent mixture. The color and hRf values of these spots were recorded by observing under ultraviolet (UV_{254nm}).

TLC study of phenols:

The powdered seeds of solanum torvum was lixiviated in methanol an rotary shaker(180thaws/min) for 24 hrs. The condensed filtrate was used for chromatography. The phenols were separated using chloroform and methanol (27:0.3) solvent mixture. The color and hRf

Values of these phenols were recorded under visible light after spraying in the plates with Folin-Ciocalteu's reagents heating at 80^0c/10 min.

TLC study of saponins:

Two grams of powdered seeds of solanum torvum was extracted with 10 ml 70% of EtOH by refluxing for 10 min. The filtrate was condensed, enriched with saturated n-BuOH, and thoroughly mixed. The butanol was retained , condensed and used chromatography. The saponins were separate using chloroform , glacial acetic acid, methanol and water(64:34:12:8)solvent mixture. The colour and hRf values of these spots were recorded by exposing chromatogram to the iodine vapours [10-14].

TLC study of sterols:

Two grams of powdered seeds of solanum torvum was extracted with 10ml methanol in water bath (80^0c /15 min). The condensed filtrate is used for chromatography. The sterols were separated using chloroform, glacial acetic acid, methanol and water (64:34: 12:8) solvent mixture. The colour and hRf values of these spots were recorded under visible light after spraying the plants with anaisaldeyde –sulphuric acid reagent and heating (100^0c/6min).

HIGH PERFOMANCE LIQUID CHROMATOGRAPHY:

HPLC analysis was carried out for the component separated in thin layer chromatography. It was performed on Schimedzu, spintrom HPLC -530 available in Science instrumentation centre, Cecri, Karikudi (TN-INDIA). The results were recorded.

ANTI-MICROBIAL STUDIES:

Preparation of inoculam :

Obtained from MTCC CULTURE. The organisms were inoculated into nutrient broth and inoculated at 37^0c for overnight. The bacterial cells were harvested by centrifuging at 5000rpm for 15 minutes. The pellet formed was washed twice with PBS and the cells were counted by haemocytometer. The bacterial cells were diluted to approximately 10^5 CFU/ml before use. The test microorganism, gram positive Staphylococcus aureus (MTCC Acc.No.7443) and Bacillus subtilis (MTCC Acc.No.441) and gram negative Escherichia coli (MTCC Acc.No.476) Salmonella species (MTCC Acc.No.53) and Pseudomonas aeroginosa (MTCC Acc.No.424) bacteria were

I.

II. Agar well diffusion method:

Anti bacterial activity of various extracts of sundakai coat was evaluated by the well diffusion method on nutrient agar medium. This was confirmed by the inhibitory effect on bacterial growth as reflected by the inhibited zone compared to known antibiotics. The sterile nutrient agar medium(20ml) in petridishes was uniformly smeared using sterile cotton swabs with test pure cultures of E.coli , vibrio cholera , streptococcus , staphylococcus aureus, bacillus substilis, klebsiella, salmonella typhimurium, salmonella cibram , proteus vulgarigus and pseudomonas.

The nutrient agar media was prepared by dissolving 0.3 beef extract, 0.3 yeast extract, 0.5 peptone, 0.5 NaCl and 1.5% agar in 1 litre of distilled water. The wells of 5mm diameter were made using sterile cork borer in each petriplates and the various extracts of sundakai fruit coat were added, a blank well loaded without test compound was regarded as control. For each treatment 10 replicates were maintained. The plates were incubated at 37^0c for 24hrs and the resulting zone of inhibition was measured by comparing control the standard antibiotic.

RESULTS AND DISCUSSION:

Phytochemical studies:

Preliminary phytochemical investigation revealed the presence of saponins, glycosides, tannins, alkaloids, volatile oils and flavonoids, as indicated in Table-1: The results showed that solanum torvum demonstrated for the presence of all phytocompounds tested, except the absence of tannins in petroleum ether extract of solanum torvum.

Antibacterial activity:

The antibacterial activities of the methanol extracts of solanum torvum and are determined against five bacteria strains. The results were compared with those produced by the standard antibiotic Tetracycline 1mg/ml. The results of the sensitivity are summarized in below the table. All strains showed sensitivity towards methanolic extract. Among the gram negative bacteria E. coli, salmonella and pseudomonas aeruginosa showed promising sensitivity 2.6 to 30mm to methanolic extract. The gram positive bacteria Bacillus subtilis and staphylococcus aureus were showed promising sensitivity toward methanolic extract. The high concentration

(100%) of solanum torvum effective against Pseudomonas aeruginosa (30.0mm) E. coli (28.2mm), Staphylococcus aureus (25.0mm), Bacillus subtilis (18.3mm), and Salmonella species(15.8mm).

Antibacterial activity that may be due to the presence of alkaloids, flavonoids, phenols, saponins and sterols, The biologically active compounds are screened by dissolving the crude powder on various compound specific solvents confirmed by the TLC. The antibacterial activity was expressed at varying concentration and dose dependent.

The various concentration of (100%,80%,60%, 40%,20%) methanol extracts of showed significant activity against all the bacterial tested. The methanol extract of solanum seeds which showed activity against all bacterial species tested at all dosages [15-24].

Table 1: Preliminary screening of secondary metabolites from solanum torvum

+ positive
_ negative

S. No	**Secondary metabolite**	**Name of the test**	**Solanum**	**torvum**
1	Alkaloids	Wagner's test	+	+
2	Flavonoids	NaOH test	+	+
3	Glycosides	Molisch test	+	+
4	Phenols	Ferric chloride test	+	+

Table 2. Rf value

S.No	**Plant name**	**Colour of spot**	**Compound**	**Rf value**
1	Solanum Torvum	Pink	Alkaloids	74.49
		Yellow	flavonoids	83.33
		Pink	glycosides	69.39
		Blue	phenols	33.33
		Light yellow	saponins	46.09
		Intense black	sterols	21.05

Table 3: TLC Profile of phytochemicals presenting in solanum torvum Phytochemical screening of the various extracts of the s.torvum fruits

Phytochemicals	**Extract**		
	Water	**Methanol**	**Ethanol**
Reducing sugar	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Tannins	-	-	-
Anthraquinones	-	-	-
Phenols	+	+	+
Flavonoids	+	+	+

Table 3: Antibacterial activity of methanol extracts from seeds of solanum torvum

S. No	**Pathogens**	**Zone of inhibition**					**Tetracycline(1mg/ml)**
		100%	80%	60%	40%	20%	34
1	E. coli	28.2	23.1	18.5	8	5.7	34
2	B. subtilis	18.3	16	14.7	10.5	4.2	22
3	S. aureus	25	17.6	15.2	9.6	3.6	20
4	salmonella.sps	15.0	13	10.1	4.1	2.6	10
5	P. aeroginosa	30	18.4	15	10.7	4.0	17

CONCLUSION

We concluded that methanolic extracts of berries of Solanum torvum swartz contain phytochemical constituents such as flavonoids, alkaloids, glycosides, saponins, tannins, sterols and phenols.The antibacterial activities of the methanol extracts of solanum torvum are determined against bacterial strains. The results were compared with those produced by the standard antibiotic tetracycline 1mg/ml. We pointed that methanolic extract have antibacterial activity.

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