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Phytochemical exploration and biological activities of stem part of Ipomoea hederifoliaL.

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Abstract:
Ipomoea hederifolia (Convolvulaceae family) haveshown long history of medicinal uses including folk remedies for many biological conditions. The current study's objectiveis to extract, isolate and characterize bioactivechemicals from the stem parts of I.hederifolia and alsomeasures the invitro antioxidant activity (using DPPH assay), antibacterial activity, total flavonoid and phenol content. Chemical examination of stem portion yieldedβ-sitosterol-3-O-β-D-glucosidecompound whose structure established on the bases of various spectral NMR data. Stempowder showed better Antioxidant activity in 30% aqeous methanol extract, more phenolic content in acetone extract and high flavonoidcontent in methanol extract(using Catechin standard)and acetone extract(using Quercetin standard), less antibacterial activity in invitro mode.
KEYWORDS: Ipomoea hederifolia; Ascorbic acid;Gallic acid; DPPH; Quercetin; Catechin.

I. INTRODUCTION

The curative nature of medicinal plants is due to the existence of numerous complex chemical compounds with various or varying compositions known as plant secondary metabolites, in plantsorgans like roots, stem, leaves, flowers also act as precussors in order to produce semi-synthetic derivatives[1].

Ipomoea hederifolia is a weak, slender twiner and it is a branched,herbaceous annual climbing vine species, vines can grow to 3-5 m long andthis therapeutic herbincluded in familyConvolvulaceae[2]. The abundant blossoming of this plant draws people attention even from far distance because of its ability to spread and flourish gregariously in the wild. It is native to southern United States,The West Indies, Mexico, Central America, South America and it also found in Asia, Africa, Australia, several Pacific islands[3]. It is adaptable and can grow without soil by providing enough nutrients and it is also known as infesting weed[4]for various crops as it has the ability to reseed itself.Itused for the treatments of ailments like stomachache(roots),intestinalparasites(tubers),inflammation(seeds) along with these, it can also act as cathartic, diuretic and expectorant [5].

Itis rich in bioactive compounds like alkaloids, acids etc. Some of the reported phytochemicals are ipanguline A, isoipanguline A, ipanguline B, and isoipangulineB[6], ipanguline B_2, ipanguline D_{10}, ipanguline D_{11}[7], palmitic acid, stearic acid, oleic acid, linoleic acid, vernolic acid, archidic acid and behenic acid [8].

This plant also shown many reported biologicalactiviteswhich includes antioxidantactivity[9],antimicrobial,anti-inflammatory,anti-convulsant and anticancer activity[10]. Even it possess potentoxytoxicactivity[11]also.

Based on above mentioned information with respect to variousreported biological activites and phytochemicals, this plant was selected for further studies like antibacterial, antioxidant total phenolic and flavonoid content activities in this experiment.

Fig.1 Ipomoea hederifolialinn.

II. MATERIAL AND METHODS

Plant collection:

The stem part of Ipomoea hederifolia was selected based on the ethano-medical information and literature survey for the present study and It was collected from CSIR-CIMAP, Research centre, Boddupal,Hyderabad. The plant was taxonomically identified and authenticated by Dr. Venkat Ramana, assistant professor, Department of Botony, Nizam college,Osmania University, Hyderabad, India. A vocherspecimen (Cimap-Ih/22) was stored at CSIR-CIMAP, Research centre, Hyderabad.

Fig.2 Driedstem of I.hederifolia

Fig.3 Course stem powder

Extraction:

Using ultrasonication equipment, 70g of stem powder was simultaneously extracted with various solvents (in order of increasing polarity) like hexane, $CDCL_3$, ethylacetate, acetone, methanol and 30% aqeous methanol for 30minutes (30min x 3 times). After filtering, all these extracts were concentrated under reduced pressure by Rota evaporator. Then estimation of antibacterial, antioxidant, total phenol and flavonoid content of all extracts was done.

Isolation:

After shade drying of stem material of plant, it pulverised Into Coarse Powder.875g of powdered stem material extracted with various solvents hexane,aqueousmethanol,and methanol by using hot percolation method. Then to attain desired concentration, all these extracts were filtered and distilled at lower pressure and accordingly calculated the percentage yield. Among these, more yield was found in methanol extract so, it was purified and packed for column chromatography using silica (100-200) which act as stationary phase and then eluted with hexane, chloroform, 50% chloroform in ethylacetate, pure ethyl acetate and acetone which act as mobile phase. In chloroform:ethylacetate(1:1) fractions, one pure white coloured compound β-sitosterol-3-O-β-D-glucosidewas yielded (30mg).

DPPH Assay Method of invitro Antioxidant Activity :

By using the DPPH radical scavenging method, the antioxidant activity of stem extracts (of various solvents) which have the capability to neutralize DPPH radicle was assessed.From the sample stock solutions (1.0 mg/mL), prepared different cocentrations like 5, 10, 15, 20, 25 and 50 µg/ml by using methanol. From each

concentrations, take 2.5ml of extract and add 1 mL of a 0.3 mM DPPH methanol solution respectively. Then this mixture was kept aside to react at room temperature for 30 minutes(incubation). At 517 nm, the sample mixture's absorbance was measured in UV and the results were compared with standard ascorbic acid absorbance values. As a negative control, 0.3 mM DPPH (1ml) and methanol (2.5ml) were combined and utilised as blank.The results were shown in [Table-2] and the percentage of antioxidant activity was determined by using the acquired absorbance values ingiven formula [12].

$$\% \text{ Inhibition of DPPH radicle} = \frac{\text{Absorbance of blank- sample absorbance}}{\text{Blank absorbance}} \times 100$$

General inference :

A sample extracts shows more antioxidant activity when its absorbance value is less than the blank absorbance value.Since blank does not have any phenolic or antioxidant compound it shows deep purple colour whereas sample contains phenolic group (low/high) that will neutralize DPPH free radicle which will reduce deep purple colour to pale yellow.

Folin - Ciocalteu assay of Total phenolic content:

By using folin- ciocalteuassay[13], the total phenolic content of Ipomoeahederifolia stem portion was estimated. In a 25ml of volumetric flask,Add aliquot (1ml) of sample extract or Gallic acid (standard solution) in different concentrations(50, 100, 150, 200, 250 and 300 mg/ lit) then add 9ml of distilled water which was deionised.Now Folin - ciocalteu phenol reagent (1ml) was added to the above mixture and shake it well then wait for 5minutes, mix 10ml of Na_2CO_3 (7%) into it, remaining volume make up with deionised distilled water. In the same way, prepare blank simultaneously without adding sample extract in it. After following 90minutes incubation period at room temperature, calculate the sample absorbance against the blank at 710nm in UV-Visible spectrophotometer [14]. The phenolic content in extracts was presented as mg gallic acid equivalents (GAE)/100 grams of fresh weight.Outcomes of all the samples that are analysed in duplicates was shown in [Table-3].

Total FlavonoidContent:

By using aluminium chloride colorimetric method[15], the total flavonoid content in various solvent extracts of I. hederifolia stem powder was established usingQuercetin and Catechin as standards. A 25 ml volumetric flask was filled with 2.5ml extract or quercetin/catechin standard solution (100,200, 300, 400, 500, and 600 mg/lit), deionised water (10ml), sodium nitrate (0.75ml , 5%) shake it for 5minutes and the next minute, add $AlCl_3$(0.75ml, 10%) and 1M NaoH (2ml) into the flask and the remaining volumefilled with deionised distilled H_2O.In the same manner, prepare blank reagent without adding sample extract. After proper mixing of both sample and blank solution, determine the sample absorbance values against prepared reagent blank at 510nm in UV. Flavanoid content in sample extracts was expressed as mg quercetin(QE) or catechin(CE) /100 grams of fresh weight[16]. Outcomes of samples were given in [Table-4].

Antibacterial Activity:

Under the clinical and laboratory standards institute guidelines[17], MIC of test molecules was determinedby using liquid broth microdilution method for evaluating antibacterial activity of test molecules under invitro condition[18].The microoraganisms employed in this investigation, P.aeruginosa PAO1ATCC15692 (gram negative), S.aureus ATCC29213 (Grampositive) and Mycobacterium tuberculosis H37Ra, were cultured to stationary phase for a period of 24 hours at 30^0C. Bacterial suspensions were made by suspending 24-hours culture in sterile normal saline and its optical density was set at 0.08 at 600nm, which equivalents to to 1.5 x 10^8 CFU/ml. The twofold serial dilutions (stem simultaneous extracts and compound-1) of test molecules which ranges from 0.06-128 µg/ml were prepared in sterile cation adjusted Muller broth (Difcolabs) in volumes of 100µl per well in 96-well U- bottom microtiter plates. In each well of the plate, 100-µl volum of inoculum (which is prepared bydiluting above mentioned bacterial suspension with respective growth medium).the final resulting inoculum(5x105 CFU/ml) plates were incubated for one day at 37^0c and then read visually.Lower concentration of compound shows no turbidity which was determined as the MIC[19].

III. RESULTS AND DISCUSSION

Chemistry:

Based on ^{1}H, ^{13}C NMR and Mass spectroscopy and previously reported literature,isolated **compound 1** frommethanol extract of I. hederifolia stem was confirmed as **β-sitosterol-3-O-β-D-glucoside** . This compound has been reported for the first time from this source (I.hederifolia stem) and this compound obtained as white amorphous powder.

^{1}H- NMR spectrum of compound1 showed chemical shift in the δH 0.79-0.96 suggesting presence of methyl protons. The proton attached to olefinic linkage was observed at δH 5.33. The proton of glucose was observed at δH 2.89-4.42 as a multiplet. The proton of CH-Group of glucoside was observed at δH 4.40. The protons of the sugar moiety showed resonance at δH 2.89 and δH 3.46-3.60 ppm.

The ^{13}C- NMR spectrum of the compound indicated 35 carbon signals of which six were for the sugar moiety and 29 attributed to the aglycone moiety. The carbon signals observed at δC 62.55(C-6'), δC 71.41 (C-4'), δC 75.07(C-2'), δC 77.87 (C-5'), δC 78.27 (C-3'), and δC102.36 (C-1') were well stable with those of glucose moiety.The signals observed at downfield δC11.94, δC 19.21 and δC21.07 were given to angular methyl group moiety associated to C-18, C-19, and C-21. The signals atδC 140.69 and δC-39.73 were assigned to the quaternary carbons at C-5 and C-13. The 1H and 13C -NMR spectra data were in line with literature reports for a polyohenol with glucose and aglycone moieties, thus, fraction compound**1** was confirmed as **β-sitosterol-3-O-β-D-glucoside**.

Fig.4chemical structure of compound – 1

Table -1: NMR data of compound-1 (**β-sitosterol-3-O-β-D-glucoside)**

POSITION	CHEMICAL SHIFT(PPM)^{13}C-NMR	MULTIPLICITY
1	36.71	CH_2
2	30.04	CH_2
3	78.27	CH_2
4	39.73	CH_2
5	140.69	C
6	121.72	CH
7	31.96	CH_2
8	31.84	CH
9	50.13	CH
10	36.71	C
11	21.07	CH_2
12	39.13	CH_2
13	42.26	C
14	56.61	CH
15	24.30	CH_2
16	28.33	CH_2
17	56.02	CH
18	11.76	CH_3
19	19.21	CH_3
20	36.18	CH
21	18.80	CH_3

22	33.99	CH_2
23	26.16	CH_2
24	45.82	CH
25	29.24	CH
26	19.77	CH_3
27	18.99	CH_3
28	23.17	CH_2
29	11.94	CH_3
1^1	102.36	CH
2^1	75.07	CH
3^1	76.96	CH
4^1	71.41	CH
5^1	77.87	CH
6^1	62.85	CH_2

DPPH Assay Method of invitroAntioxidant Activity:

This Assay assesses the capability of I. hederifolia stem extracts to convert the unpaired electrons into paired once, so reducing DPPH radicle to the equivalent hydrazine. According to the current study's dose-dependent analysis of DPPH radical inhibition[20], extracts reduce DPPH radical levels. The percentage of DPPH radicals that were scavenged at different concentrations (using various solvents of stem extract) is shown in (Table-2) and serves as an example of how different extracts affect DPPH radicals.

Table-2: various solvent extracts of I. hederifoliastem on DPPH radicals.

Concentration (µg/ml)	Hexane (%)	chloroform (%)	Ethyl Acetate (%)	Acetone (%)	Methanol(%)	30% Aqeous Methanol (%)	Ascorbic Acid(%)
05	0.487	2.682	19.163	35.797	23.693	22.879	56.26
10	0.731	0.121	20.557	46.303	35.54	32.103	58.56
15	1.951	9.878	26.829	52.362	47.387	22.509	74.58
20	1.707	2.682	25.783	81.26	64.111	44.649	93.92
25	3.048	7.56	50.174	76.267	80.487	57.195	94.32
50	0.121	5.121	51.219	82.26	82.257	83.015	97.23

According to the data(Table-2),30% aqeous methanol extract has shown more antioxidant activityamong all,by inhibiting DPPH free radicle levelsat 50µg/ml concentration (83.015%) with respective to ascorbic acid (97.23%) which used as standard. Overall the acetone and methanol extracts also shown better antioxidant activity.

Total Phenolic Content:

Total phenolic content of stemportion of I. hederifolia which simultaneously extracted with various solventshexane, $CDCl_3$, ethylacetate, acetone, methanol and 30% aqeous methanol was equivalent to 17.687, 29.029, 54.004, 61.415, 24.767 and 33.617mg respectively as Gallic acid equivalents/gram of extract and is depicted in (Table–3).

Table-3: Gallic acid equivalents (GAE) of various solvent extracts of I. hederifolia stem.

S.no	extract	Unknown concentration(µg/ml)	mg/100gramsgallicacid equivalents
1	Hexane	176.84	17.684
2	Chloroform	290.29	29.029
3	Ethylacetate	540.04	54.004
4	Acetone	614.15	61.415
5	Methanol	247.67	24.767
6	30%Aqeous methanol	336.17	33.617

Among all simultaneous extracts, acetone extract of I. hederifolia stem contains greater phenolic content (61.415 mg GAE/gr. Ext.).

Order of Phenolic content of plant I. hederifolia stemextract
Acetone > Ethylacetate > 30%Aq.Methanol > Chloroform > Methanol > Hexane

Total Flavanoid Content:

Two standards Quercetin and catechin were used to determine total flavonoid content.Using Quercetin standard , the total flavonoid content in I. hederifolia stem which is simultaneously extracted with various solvents hexane, chloroform, ethylacetate, acetone, methanol and 30% aqeous methanol was found to be 5.730, 46.849, 59.311, 151.668, 88.596 and 79.437 mg/100grams equivalents respectively.Whereas using catechin standard, total flavonoid content for the abovementioned extracts was found to be 60.764, 62.408, 67.074, 61.048, 79.876 and 75.939 mg/100 grams equivalents respectively and results of quercetin and catechin wereshown in (Table-4).

Table-4: mg/100 grams equivalents of Quercetin and Catechin of stem extracts of I. hederifolia

Extracts	Quercetin		Catechin	
	unknown concentration (µg/ml)	mg/100grams Quercetin Equivalents	Unknown concentration (µg/ml)	mg/100grams Catechin Equivalents
Hexane	57.30	5.730	607.64	60.764
Chloroform	468.49	46.849	624.08	62.408
Ethylacetate	593.11	59.311	670.74	67.074
Acetone	1516.96	151.668	610.48	61.048
Methanol	885.96	88.596	798.76	79.876
30% Aqeous. Methanol	794.37	79.437	759.39	75.939

Using Quercetin as a standard reference, Simultaneous acetoneextract(151.668 mg QE/gr. Ext.)shown more flavonoid content compare to other extracts. In the case of catechin standard, methanol extract (79.876 mg CE/gr.Ext.) shown highest flavonoid content compare to remaining extracts.

Order of total flavonoid content of different simultaneous extracts of stem
In case of Quercetin:
Acetone > Methanol > 30% Aq.Methanol> Ethylacetate > Chloroform > Hexane
In case of Catechin :
Methanol >30%Aq. Methanol > Ethylacetate > Chloroform > Acetone > Hexane

Antibacterial activity :

a) Gram positive bacteria: streptococcus aureus

Compare to standard Ciprofloxacin(0.06 µg/ml), Zone of inhibition of simultaneous extracts and isolated compound of Ipomoea hederifolia stem which employes Streptococcus aureus against gram positive bacteria had shown no significant action.

b) Gram negative bacteria: pseudomonas aeruginosa

using pseudomonas aeruginosa, zone of inhibition of simultaneous extracts and isolated compound less activity against Gram positive bacteria compare to standard ciprofloxacin (0.25µg/ml).

c) Tuberculosis - Mycobacterium tuberculosis

Usingthisbacteria, the simultaneous extracts and isolated compound of I. hederifolia stem showed noactivityagainst gram positive bacteria in comparisionto reference standardsIsoniazid (0.015µg/ml) and Rifampicin (0.0019µg/ml) and the results were expressed in (Table-5).

Table-5: zone of inhibition of different extracts and compound of I. hederifolia stem.

Extractcompound	p. aeruginosa PAO1ATCC15692 (Gram- negative) MIC (µg/ml)	S. aureus ATCC29213 (Gram- positive) MIC (µg/ml)	Mycobacterium tuberculosis H37Ra MIC (µg/ml)
Hexane	>128	>128	>128
Chloroform	>128	>128	>128
Ethylacetate	>128	>128	>128
Acetone	>128	>128	>!28
Methanol	>128	>128	>128
30%Aq. Methanol	>128	>128	>128
Compound (IHSM-1)	>128	>128	>128
Ciprofloxazin	0.06	0.125	----

Isoniazid	----	----	0.015
Rifampicin	----	----	0.0019

IV. SUMMARY AND CONCLUSION

From the overall studies, extraction and isolation of stem part of I.hederifolia resulted in the separation of one compound whose structure confirmed as **β-sitisterol-3-O-β-D-glucoside** based on Mass and NMR spectrometry. This compound has been reported for the first time from the stem part of Ipomoeahederifolia. While the Biological activity of simultaneousextractsof I. hederifolia stem shown that 30% aqeous methanol extract have more antioxidant activity (DPPH Method) at the concentration of 50µg/ml with 83.012% inhibition rate of free radicle. using Gallic acid as a standard, the total phenolic content (Folin-Ciocalteu assay method) was more in acetone extractwith 61.415 mg GAE/gr. Ext. Using aluminium chloride colorimetric method, the total flavonoid content was high in acetone extract (151.668 mg QE/Ext.) in case of Quercetin Standard. Whereas using Catechin as standard, methanol extract showedmore flavonoid content(79.876 CE/gr.Ext.).The stem part of I. hederifoliaplant didn'tshowed significant antibacterial activity but it shown better antioxidant activity and also high phenolic and flavonoid content in it.

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