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Studies on Pharmacological and Chemical Composition of Crude Plant Extract of *Rivea Hypocrateriformis*

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ABSTRACT

The aim of the study was to assess Pharmacognestic study of crude plant extract of *Rivea hypocrateriformis* and was carried out to characterize the chemical composition of some constituents by GC-MS analysis. Different solvent extracts (aqueous, methanolic, chloroform, ethyl acetate and DMSO) of plant *R. hypocrateriformis* leaves were assessed for in vitro antimicrobial activity assay by disc diffusion method furthermore antioxidant assay was carried out DPPH free radical scavenging activity, Phytochemical screening was carried out by 'guide to modern techniques of plant analysis" and GC-MS analyses were performed to identify the constituents present in the plant that stand behind such activities. Due to higher polarity, DMSO extract show revealed presence of maximum phytochemical composition susceptibility as well as methanol and chloroform shows average amount of phytoconstituents. The antibacterial screening is the major of the inhibition hollow observed in inhibition zone. The highest inhibition zone was observed in DMSO extract against each bacterial strain. Where E. coli shows mid active zone inhibition and S. aureus show less, IC_{50} value of the sample was found to be moderate as compared to standard and the eight compounds were identified in *R. hypocrateriformis* leaf extract by GCMS analysis. *R. hypocrateriformis* plant had considerable major chemical composition present in crude extract. Due to presence of major chemical components make it seems to be important for medical purposes and plant contains Potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments. Also plant extracts can be used for the treatment of infections caused by the strains of the test bacterial organisms.

Keywords: Antimicrobial, DPPH, GC-MS.

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INTRODUCATION

Natural compounds of plants are derived from the phenomenon of biodiversity. Plants produce these compounds to enhance their survival and competitiveness. Due to their biological activities, many plant-derived compounds are used for the treatment of human illnesses or diseases. A World Health Organization (WHO) survey indicated that about 70–80% of the world's population relies on traditional medicine based mainly on plant materials for their primary healthcare[1]. The benefits of individual phytochemical supplements are largely unproven. Furthermore, they are not regulated. High concentrations of some phytochemical may behave like drugs and be toxic, possibly even contributing to cancer cell growth. *Rivea hypocrateriformis* (Desr.) choisy belongs to family convolvulaceae phys. It is extensively perennial, robust, twining climber reaches up to 2-3m in height and woody at the base. Stem terete, more or less silky – pubescent, become woody, glabrous, with the maturity, leaves alternate, 3-10 cm long and as much as broad, orbicular, obtuse, rarely mucronulate glabrous above, silly pubescent beneath, base chordate, petioles 3-5 cm long silky. Flowers white in 1-3 –flowered, peduncles very short. Glabrous stemens-5, included anthers linear oblong[2]. *R. hypocrateriformis* is an effective agent in anti-implantation and pregnancy interruption in female albino rats[3]. This plant is used as psychoactive drug. Other species of the same family such as *R. corymbs Hall* and *Ipomeas violate L*. found in Mexico, where Indians use these plants as psychedelic drug[4]. Juice of leaves of *R. hypocrateriformis* is taken with cow's milk in rheumatic pain and also the leaves extract is used for skin disease of hair scalp[5]. It has been reported that leaves, stem, and flowers extracts of *R. hypocrateriformis* show strong antioxidant activity[6].

MATERIALS AND METHOD

Collection of sample:

Fresh leaves of *R. hypocrateriformis* were collected from Mardi village, Dist. Amravati (Central region of India) in the month of January-2014. The plant were authenticated by a taxonomist from Department of Botany ACS College Amravati. Fresh leaves were washed well using tap water and twice using distilled water and it was dried in shade for a period of 20-25 days, at an ambient temperatures of 29°C. After drying, plants were cut into small pieces. The dried sample was grind properly using a mixer to obtain the powdered form.

Chemicals and microbial cultures

All the chemicals and standard antibiotics used in this work were purchased from Sigma Aldrich, Merck and Hi-media, Mumbai, India. The reference bacterial strains used in this study were

obtained from American Type Culture Collection (ATCC) and Microbial Type Culture Collection (MTCC) Institute of Microbial Technology, Chandigarh, India.

Preparation of plant extract

The plant were dried over ambient temperature and the dried samples were grind properly and dried powder sample was extracted in Methanol at 65ºC, Chloroform 61ºC, Dimethyl sulphoxide 189ºC, Ethyl acetate 77ºC and Distilled water 100ºC by using Soxhlet apparatus[7]. Further extracts were concentrated by gradually evaporating the respective solvent on hot water bath. The concentrated extract was collected in sterile bottles and kept in a cool and dark place prior to analysis.

Phytochemical Screening

All crude extracts of *R. hypocrateriformis* were diluted in their respective solvents and subjected for qualitative preliminary Phytochemical screening to identify the presence of the secondary metabolites according to the standard methods[8]. From the intensity of the color inferred for the tests, they were rated for their presence.

Antimicrobial Activity of *R. hypocrateriformis* leaves

Antimicrobial activity of five organic extracts viz. aqueous, methanolic, chloroform, ethyl acetate and DMSO of *R. hypocrateriformis* leaves were determined by Agar disc diffusion assay according to the Manual of antimicrobial susceptibility testing[9]. To assay the various antibiotics for bactericidal activity against test strains, *E. coli, K. pneumoniae, P. aeruginosa, S. aureus, P. acnes, S.typhi* were spread on separate nutrient agar plates. All five organic extracts (20 µl,) were loaded separately on sterile discs (6 mm diameter), allowed to dry and placed on the bacteria inoculated nutrient agar media. Negative control was prepared by loading the discs with solvents and positive control was by tetracycline. The plates were incubated at 37°C for 24 hr and zone of inhibition around the disc were measured. The experiment was done with three replicates for consistency of the experiment. The organic extracts, DMSO and Methanol, exhibiting maximum antimicrobial activity, were selected for subsequent studies.

Antioxidant activity of *Rivea hypocrateriformis*

The radical scavenging activity of the plant extracts against 2,2 Diphenyl -1-Picryl hydroxyl radical were determined by UV visible spectrophotometer carry 60 (Agilent). Antioxidants present in plant were quantified employing folin's reagent i.e. DPPH. DPPH assay is often used to evaluate the ability of antioxidant to scavenge free radicals which are known to be a major factor in biological damage caused by oxidative stress[10].

Antioxidant activity (DPPH free radical scavenging activity) of methanolic extract

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2, 2-diphenyl-1- picrylhydrazyl (DPPH) free radical was determined by the method described by shen *et al*[11]. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1000-5000 µg/ml solution. 3.94 mg of DPPH was prepared in 100 ml methanol and 2.96 ml of this solution was mixed with 40 µl of sample solution and standard solution separately. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using UV-Vis Spectrophotometer (carry 60 Agilent). DPPH solution was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below % of DPPH Radical Scavenging activity % RSA.

Abs control - Abs sample / Abs control ×100

Abs control is the absorbance of DPPH radical and methanol. Abs sample is the absorbance of DDPH radical + sample extract was the measure. Absorbance values were corrected for free radical decay using blank solution. And IC_{50} value can calculate by using calibration curves verses percentage of inhibitions.

GC-MS analysis of *R. hypocrateriformis*

Gas Chromatography

Gas chromatography of the plant extract was carried out on a 6890 gas chromatography model 5765 with direct injector and split ratio set to 10:1. (DB-5) (5% phenyl polysioxane, 30m length 250u internal diameter; 0.25 um film coating) fused capillary column. Helium was the carrier gas at 1.0 ml min. The oven temperature program was programmed to start at 35 ^{o}hold for 2 min then temp at 20^{o}C per min to 300^{o}C and hold for 5 min. Injector and detector temperature were 220 ^{o} and 230^{o} respectively. Injection size was 0.02 ul. Fig 3 shows gas chromatogram of plant *R. hypocrateriformis* leaves

Gas Chromatography and Mass Spectroscopy

A JEOL GC-mate II bench top double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000^{1} software was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 700 at 0.3 seconds per scan with a 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 750 at 1 second per scan.

Identification of chemical constituents

Identification of the chemical constituents was done on the basis of retention index (RI) using a mass spectra library search NIST and by com-paring the mass spectral and retention data with literature[12]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

Phytochemical evaluation is to confirm the presence of various chemical constituent present in plant. Screening of Phytochemical was done according to the polarity of five organic extracts. viz. aqueous, methanol, ethyl acetate, chloroform and DMSO. Phytochemical analysis is listed in Table 1. Due to higher polarity of DMSO extract show presence of maximum phytochemical composition substeptibility as well as methanol and chloroform show in average amount of phytoconstituents and these phytoconstituents independently are responsible for the broad range of medicinal properties of *R.hypocrateriformis.*

Table 1: Phytochemical Screening of *Rivea hypocrateriformis* leaves extracts

Sr. No.	Phytochemicals	Test Perform	AQE	EAE	CLE	MEE	DMSOE
1	Steroid	Ring Test	+	++	++	++	++
2	Tannins and Phenolic Compounds	Ferric Chloride	++	_	+	++	+
		Lead Acetate	++	_	+	++	+
		Gelatine	_	_	_	_	_
3	Terpenoids	Salkowski	+	+	+	+	+
		Mayer test	_	_	_	_	_
4	Alkaloids	Dragendorff's	+	++	++	++	++
		Wagner Test	++	++	_	++	_
		Ethyl acetate	_	_	_	++	+
5	Flavonoids	Alkaline Reagent	_	_	_	_	_
6	Cumarine	Fluorescence	++	++	+	_	_
7	Cardiac Glycosides	Legal Test	_	_	_	_	_
8	Phytosterols	Libermann Buchard Test	+	+	++	++	+

++ indicates: strong presence, + indicates: weak presence, - indicates: strong absence

The antibacterial screening was carried out for five organic solvent plant extract. Antibacterial activity of plant *R. hypocrateriformis* is listed in Table 2.The antibacterial screening the highest inhibition zone was observed in DMSO extract in each bacterial strain where *E.coli* showed mid active zone of inhibition where *S. aureus* show less. The same observations were noted in case of solvents methanol and chloroform while aqueous and ethyl acetate extracts showed minimum inhibition zone in all bacterial strains viz. *S.typhi, E.coli, P.aeru, K.pneu.*

Table 2: Antimicrobial activities of leaves different solvents of *R. hypocrateriformis*

Test bacteria	Zone of inhabitation (mm)					Standard
	Test Samples					
	AQE	MEE	CLE	EAE	DMSOE	AB
S.aureus	09±0.2	11±0.5	08±0.5	11±0.3	12±0.5	24±0.3
E.coli	18±0.3	13±0.5	10±0.6	00	14±0.3	22±0.4
P.aeruginosa	11±0.4	12±0.3	12±0.6	09±0.3	13±0.3	23±0.2
K. pneumoniae	09±0.3	14±0.3	15±0.4	11±03	14±0.3	25±0.5
S.typhi	10±0.3	13±0.3	12±0.5	10±0.3	15±0.3	28±0.4

Values are represented as the mean ±S.D of experiments.

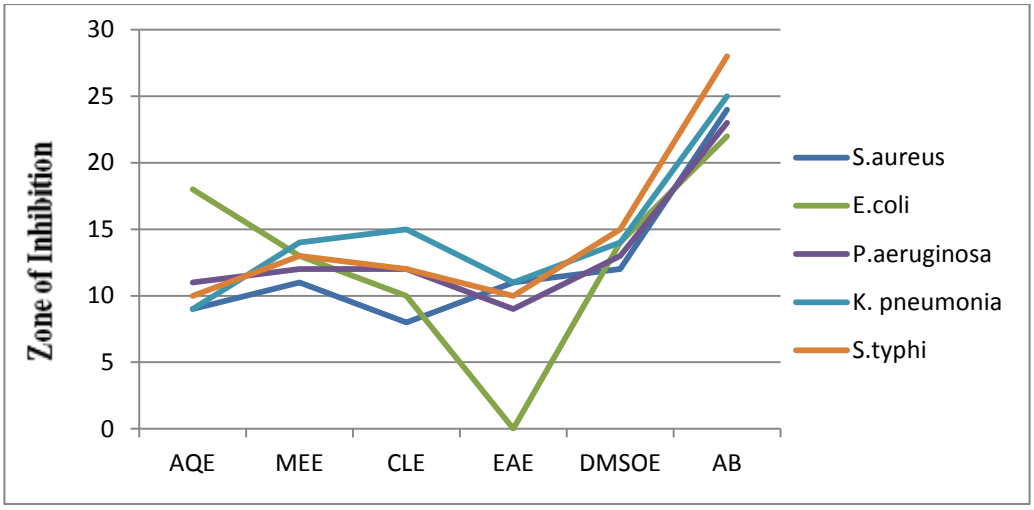

Figure 1: Mean zone of inhibitation of five organic extracts of *R. hypocrateriformis*

The radical scavenging activity of R. hypocrateriformis leaf extract was tested using stable free radical DDPH (Deep purple colour) as DPPH has the advantage of being unaffected by certain side reaction Figure 2 show the DDPH radical scavenging activity of Rivea extract with ascorbic acid as reference where the IC_{50} value for the Rivea extract (IC_{50} = 4147) and IC_{50} value was found to be moderate as compared to standard (IC_{50}= 2782)

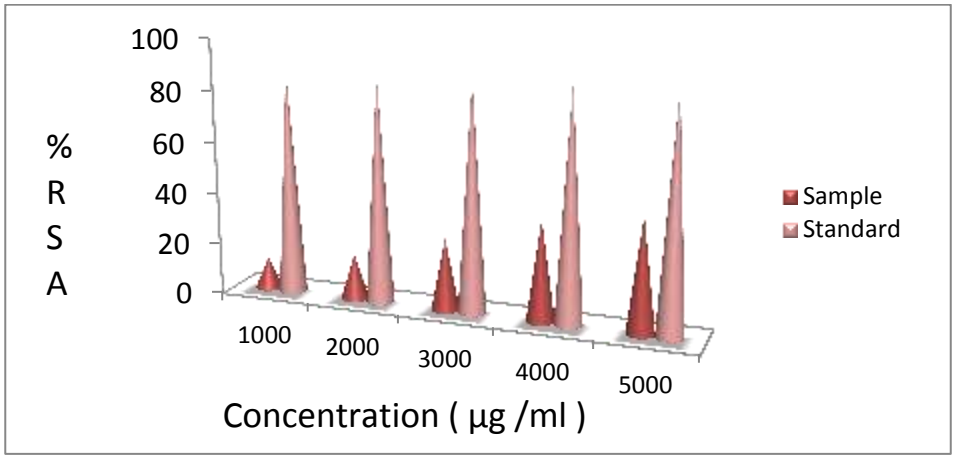

Figure 2: DDPH radical scavenging activity of *R. hypocrateriformis*

Interpretation on mass spectrum GC-MS was concluded using the data base of National Institute Standard and Technology (NIST).The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Total eight compounds were identified in *R. hypocrateriformis* leaf extract by GCMS analysis which are presented in Table 3. The active principle with their retention time (RT), molecular formula, molecular weight and peak area or concentration (%) were taken into account. The major chemical constituents identified in leaves extract of *R. hypocrateriformis* were 4,25-Secobscurinervan,21-deoxy-16-methoxy-22-methyl (10.64%), [-(+)-Ascorbic acid 2,6-dihexadecanote (15.12%), Cis,trans-5,{9-Cyclododecadiene-cis-1,2-diol(19.84%), Oleic Acid (8.87%), Rescinnamine (11.19%), Estra-1,3,5(10)-trien-17a-ol (16.55%), Pregn-5-ene-3,11-dione,17,20;20,21-bis[methylenenbis(oxy)]-cyclic 3-9 (1,2-ehanediyl acetol) (13.41%), Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1'4,4'-tetrone (04.38%), Plant extract of *R. hypocrateriformis* contains chemical components showing interesting biological properties due to presence of some alkaloids like Rescinnamine and Estra-1, 3, 5 (10)-trien-17á-ol are steroid compound, Some authors stated that the compound Estra-1, 3, 5 (10)-trien-17á-ol is a steroid but differs from estradiol, a sex hormone, in the absence of an OH group at C3. Steroids, though similar in basic structure, have extreme specificity[13] hence the steroid in the essential oil cannot be said to function like estradiol.and Rescinnamine is an antihypertensive, tranquillizer drug[14].

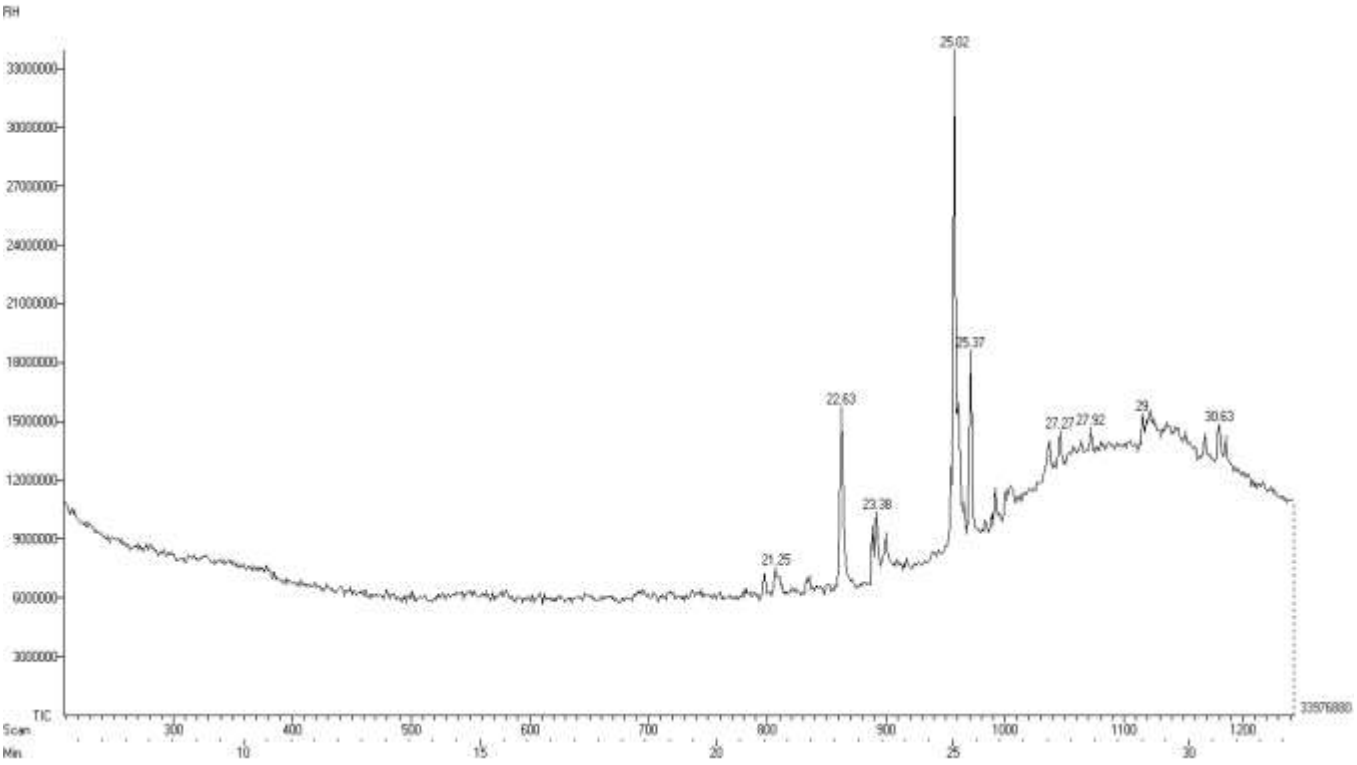

Figure 3: Gas chromatogram of leaves extract of *R. hypocrateriformis*

Table 3: Chemical composition of *R. hypocrateriformis* leaves

Sr. No	Retention Time	Name of chemical constituent	Molecular Formula	Molecular Weight	Peak Area %
1.	21.25	4,25-Secobscurinervan,21-deoxy-16-methoxy-22-methyl(22a)	$C_{23}H_{32}N_2O_2$	368.51	10.64
2.	22.63	[-(+)-Ascorbic acid 2,6-dihexadecanote	$C_{38}H_{68}O_8$	652.049	15.12
3.	23.38	Cis,trans-5,{9-Cyclododecadiene-cis-1,2-diol	$C_{12}H_{20}O_2$	169.29	19.84
4	25.02	Oleic Acid	$C_{18}H_{34}O_2$	282.26	8.87
5	27.27	Rescinnamine	$C_{35}H_{32}N_2O_9$	634.29	11.19
6	27.92	Estra-1,3,5(10)-trien-17a-ol	$C_{18}H_{24}O$	256.38	16.55
7	29	Pregn-5-ene-3,11-dione,17,20;20,21-bis[methylenenbis(oxy)]-cyclic 3-9(1,2-ehanediyl acetol)	$C_{25}H_{34}O_7$	446.23	13.41
8	30.63	3,8,8-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1'4,4'-tetrone	$C_{27}H_{21}NO_7$	471.46	04.38

Oleic acid has been found to be fungi static against a wide spectrum of moulds and yeasts. For example, it was observed to cause a delay of 6-8 h in the germination of fungal spores, and was also found to be effective at concentrations as low as 0.7% v/v[15]. It has also been proposed that these fatty acids have potential antibacterial and antifungal principle for clinical applications[16], Oleic acid, the known tumor promoter 12-O-tetradecanoylphorbol- 13-acetate activated protein kinase C from rat brain[17] and another use of oleic acid is that it finds great use in preserving foodstuffs including bakery products like cakes and pastries with sweet soft cream as a topping which are prone to quick spoilage under conditions of non-refrigeration and Ascorbic acid is required for collagen synthesis and plays a structural role in bone, cartilage and teeth. It is also essential for the oxidation of the amino acids phenylalanine and tyrosine, and the conversion of folacin to tetrahydrofolic acid.

CONCLUSION

Finding indicates that *R.hypocrateriformis* growing in India has considerable major chemical composition present in crude extract due to which it seems highly important for medic purposes. Chemical compound like alkaloid (Rescinnamine) which was found in plant extract show numerous pharmacological activities like antimicrobial, analgesic, antihypertensive and compound like Estra-1, 3, 5 (10)-trien-17á-ol are steroid compound increase a sex hormone, Oleic acid is found to be fungi static against moulds and ascorbic acid which act as vitamin source which are found in plant extract *R. hypocrateriformis*. Hence it is concluded that *R. hypocrateriformis* has greater applications of medicinal property. Also the plant contains potential antibacterial

components that may be useful for evolution of pharmaceutical for the therapy of ailments and also plant extracts can be used for the treatment of various bacterial infections.

ABBREVIATIONS

Aqueous extract [AQE], Methanolic extract [MEE], Chloroform extract [CLE], Ethyl acetate extract [EAE], Dimethyl sulphoxide extract [DMSOE], Antibiotic [AB], Standard deviation [S.D], 2,2 Diphenyl -1-Picryl hydrazyl [DPPH], Inhibition Concentration [IC_{50}], radical scavenging activity [RSA], National Institute Standard and Technology [NIST], Flame ionization detector [FID]

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