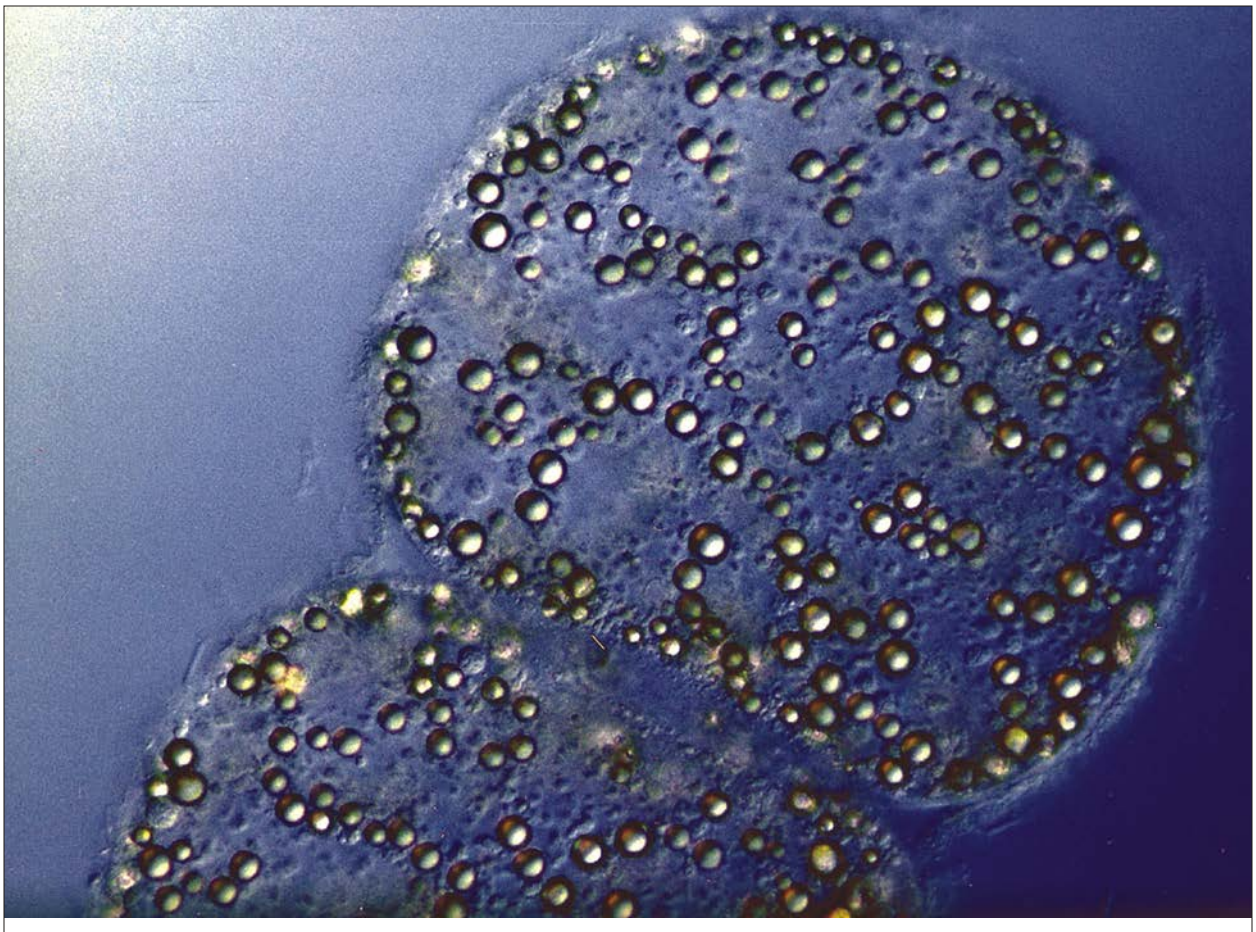

Microbiology

Journal

ISSN 2153-0696

Academic
Journals Inc.

www.academicjournals.com

Microbiology Journal 4 (1): 22-26, 2014
ISSN 2153-0696 / DOI: 10.3923/mj.2014.22.26

Antimicrobial and Phytochemical Analysis of *Revia hypocrateriformis*

[1]S. Saboo, [2]G.G. Tapadiya and [1]S.S. Khadabadi
[1]Department of Pharmacognosy, Government College of Pharmacy, Amravati (MS), India
[2]R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur (MS), India

Corresponding Author: G.G. Tapadiya, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Karwand Naka, Shirpur (MS), India

ABSTRACT

Revia hypocrateriformis (RH) belonging to family Convolvulaceae is an important medicinal plant, traditionally used for various diseases. Present study aims to investigate antifungal and antibacterial potency along with phytochemical analysis. These activities had been checked against five gram positive and gram negative bacteria and three fungi. Phytochemically it contains phenolics, catechin and gallic acid. Plant have proved to be significant natural resources as effective antimicrobial and chemotherapeutic agent and offer a broad spectrum of activity with greater emphasis on preventive action. These findings show that the crude extract of plant can be used as prophylactic agent in many diseases.

Key words: *Revia hypocrateriformis*, antibacterial, antifungal, gram negative, positive strain

INTRODUCTION

Eight decades after the discovery of penicillin, the intense efforts to find new and more effective antimicrobial agents continue. One of the main causes is attributed to the misuse of antibiotics which has led to the development of resistance in pathogens (Demain and Sanchez, 2009). Faced with this urgent need, different strategies are being used where microbial products continue to be a major source of new natural models to study (Duru and Onyedineke, 2010).

The investigation of certain plants for their antimicrobial properties may yield useful results. Researchers are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for the antimicrobial properties (Aibinu *et al*., 2007; Ndukwe *et al*., 2007).

Revia hypocrateriformis (RH) is climbing shrub known for a large number of biological activities such as antibacterial, antidiabetic, antiimplantation, in treatment of burning and piles, antidepressant, anticancer and analgesic properties (Kirtikar and Basu, 1935; Dhawan *et al*., 1980; Shivalingappa *et al*., 1999). Chemically it known to contains amino acid and sugar (Dhore *et al*., 2001). Due to its ethnopharmacological importance and literature survey reveals lack of any systematic biological and bio-chemical investigation of this plant. The present investigation sought to obtain data on antibacterial potential of RH. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery.

MATERIALS AND METHODS

Microorganisms: Microbial species, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10535), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Candida albicans* (ATCC 10231), *Aspergillus flavons* (ATCC 15517) and *Aspergillus niger* (ATCC 16404) from the stock cultures of microorganisms (Department of Microbiology, Amravati University) were used. Sabouraud 2% (w/v)-glucose agar, Muller-Hinton agar and Muller-Hinton broth were supplied by Merck (Germany) and RPMI 1640 broth by the Institute of Immunology.

Herbal material and extraction: The aerial parts of RH were collected in the month of August-September 2011 from Amravati District, Maharashtra and were authenticated by Prof. Dr. Bhowagaokar, VIHS, Amravati, Maharashtra, India. A voucher specimen (AMT-36) has been preserved for future reference. Extraction of fine to coarse plant material was done with different polarities of solvents, petroleum ether (PE), chloroform (SCH), ethanol (SEE) and water (SAE). The mixtures were then filtered and evaporated to dryness under reduced pressure (17 mm Hg, 45°C, 10-20 min depending on the solvent).

Phytochemical analysis: This extraction scheme in brief, yielded non-polar [petroleum ether (60-80°) m], less polar (chloroform) and highly polar (ethanol and aqueous) extracts and presence of different classes of secondary metabolites including flavones, flavonoids, phenolics, quinones, saponins and triterpenoids were confirmed using conventional phytochemical tests.

Antibacterial susceptibility testing: A disk diffusion method, according to NCCLS (1997a) and Saboo *et al.* (2013), was employed for the determination of antibacterial activity of the extracts. Inoculums were prepared with fresh cultures of microbial strains, cultured on Sabouraud 2% (w/v) glucose agar for 18 h (48 h for fungi) at 37°C with saline. All agar plates were prepared in 90 mm Petri dishes with 22 mL of agar, giving a final depth of 4 mm. One-hundred microlitres of a suspension of the tested microorganisms (108 cells mL^{-1}) were spread on the solid media plates. Sterile filter paper disks were impregnated with 50 µL (10 mg mL^{-1}) of the extracts of RH placed on inoculated plates. These plates, after standing at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the fungi. Standard disks of Tetracycline (10 mg mL^{-1}) were used individually as positive controls. The diameters of the inhibition zones were measured in millimeters (to the nearest 0.1 mm) using Antibiotic Zone Reader. Each test was performed in quintuplicate and repeated three times.

Antifungal assay: Anti-fungal study was carried out through the same procedure as for the antibacterial study except that Sabouraud dextrose agar media (SDA MEDIUM) was used. Ketaconozol was used as a standard.

Minimal inhibitory concentration: Minimal Inhibitory Concentration (MIC) was determined by the twofold micro dilution method in Muller-Hinton broth for bacterial

strains and RPMI 1640 for yeast and mould according to the Clinical and Laboratory Standards Institute (formerly NCCLS) M-27A recommendations (NCCLS, 1997b).

RESULTS AND DISCUSSION

The phytochemical screening revealed the presence of higher concentration of secondary metabolites including flavonoids, alkaloids, steroid and phenolic compound (Table 1 and 2) as confirmed by phytochemical tests and analysis. These bioactive components are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the antimicrobial property to plants (Lutterodt *et al*., 1999). The antimicrobial potency was assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters and MIC values. The zone of inhibition of extracts ranged from 10 to 29 mm (Fig. 1). The MIC was found in the range of 5 to 0.625 mg mL^{-1} (Table 3). The results showed that, SEE extract was more active against gram positive bacteria, *S.aureous* and *B. subtilis* whereas aqueous extract had strong inhibitory effect against gram negative bacteria, *E.coli*, *P. aerugenosa*, *P. vulgaris* and fungi, *A. flavons*, *A. niger* and *C. albicans* as indicated by lower MIC value (Table 3) . The petroleum ether and chloroform extract also had inhibitory effect on tested organism but less when compared to ethanol and aqueous extracts.

Table 1: Phytochemical study of RH extracts

Tests	PE	SCH	SEE	SAE
Carbohydrates	-	-	-	+
Cardiac glycosides	-	-	+	-
Saponin glycosides	-	-	-	-
Steroids	+	+	-	-
Fatty acids	+	-	-	-
Alkaloids	-	+	-	-
Flavonoids	-	-	+	+
Tannins	-	-	+	+
Mucilage	-	-	-	-
Resin	-	-	-	-

PE: Petroleum ether extract, SCH: Successive chloroform extract, SEE: Successive ethanol extract, SAE: Successive aqueous extract

Table 2: Results of quantitative estimation of phytoconstituents

Phytochemical constituent	Extract	RH* (%)
Carbohydrate	SAE	16.11±0.23
Protein	SAE	10.98±0.11
Alkaloid	SCH	15.8±0.030
	SEE	7.34±1.12
Total phenolic	SEE	15.10±0.25
	SAE	13.81±0.32
Steroid	SCH	9.41±0.11
	SEE	5.10±0.28
Flavonoid	SEE	42.39±0.14

*Values are Mean±SD (n = 3)

Table 3: MIC values of RH extracts

	Gram positive		Gram negative			Fungi		
Extract (μg mL^{-1})	*S. aureus*	*B. subtilis*	*E. coli*	*P. aerugenosa*	*P. vulgaris*	*A. niger*	*C. albicans*	*A. flavon*
AE								
10.000	-	-	-	-	-	-	-	-
5.000	-	-	-	-	-	-	-	-
2.500	++	+	-	+	+	-	-	-
1.250	+++	++	+	++	+	+	+	+
0.625	+++	+++	++	++	++	++	++	++
ME								
10.000	-	-	-	-	-	-	-	-
5.000	-	-	-	-	-	-	-	-
2.500	-	-	+	+	-	+	-	-
1.250	+	+	+	++	++	++	+	+
0.625	+	+	++	+++	+++	++	++	++
TC								
10.000	-	-	-	-	-	*	*	*
5.000	-	-	-	-	-	*	*	*
2.500	-	-	-	-	-	*	*	*
1.250	-	-	-	+	-	*	*	*
0.625	+	+	+	++	+	*	*	*
KC								
10.000	*	*	*	*	*	-	-	-
5.000	*	*	*	*	*	-	-	-
2.500	*	*	*	*	*	-	-	-
1.250	*	*	*	*	*	-	+	-
0.625	*	*	*	*	*	+	++	+

SEE: Successive ethanol extract, SAE: Successive aqueous extract, TC: Tetracyclin, KC: Ketaconozol, -: No growth, +: Less growth, ++: Moderate growth, +++: Heavy growth and *: Not performed

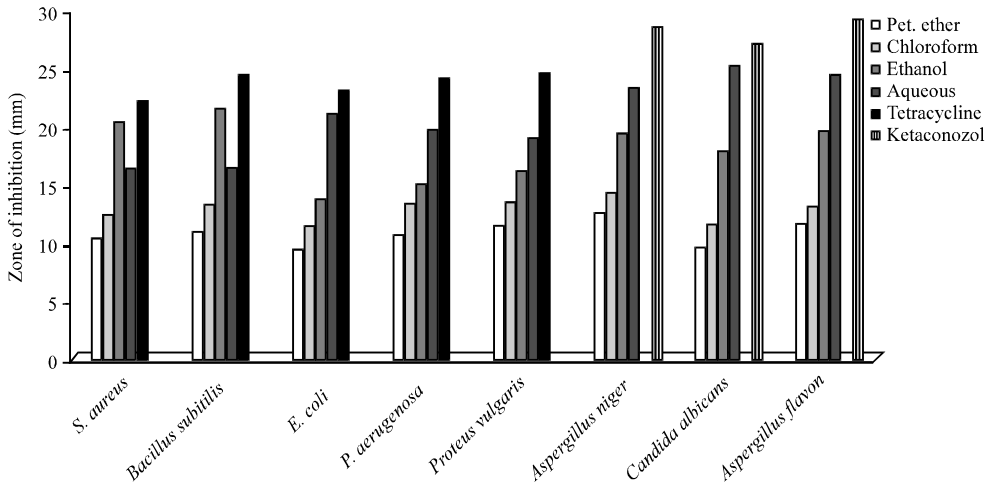

Fig. 1: Zone of inhibition of RH

CONCLUSION

The overall results of this study indicated that the antimicrobial activity of the different crude extract from RH could be due to the presence of secondary metabolites, antibacterial and antifungal

compounds. To the best knowledge, the biological activity of this plant against bacteria and fungi is reported for the first time. The present investigation provides important baseline information for the use of *Revia hypocrateriformis* as well as its constituents for the treatment of infections associated with the studied microorganisms.

REFERENCES

Aibinu, I., T. Adenipekun, T. Adelowotan, T. Ogunsanya and T. Odugbemi, 2007. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. Afr. J. Trad. Compl. Altern. Med., 4: 185-190.

Demain, A.L. and S. Sanchez, 2009. Microbial drug discovery: 80 years of progress. J. Antibiot., 62: 5-16.

Dhawan, B.N., M.P. Dubey, B.N. Mehrotra, R.P. Rastogi and J.S. Jandon, 1980. Screening of Indian plants for biological activity: Part IX. Indian J. Exp. Biol., 18: 594-606.

Dhore, M.N., B.U. Cochhi and J.A. Tidke, 2001. Amino acid and sugar from floral nector from some local plants in India. J. Phyotol. Res., 13: 171-174.

Duru, C.M. and N.E. Onyedineke, 2010. *In vitro* study on the antimicrobial activity and phytochemical analysis of ethanolic extracts of the mesocarp of *Voacanga africana*. Am. J. Plant Physiol., 5: 163-169.

Kirtikar, K.M. and B.D. Basu, 1935. Indian Medicinal Plants. 2nd Edn., Vol. 2, International Book Distributors, Delhi, India.

Lutterodt, G.D., A. Ismail, F. Basheer and H.M. Baharudin, 1999. Antimicrobial effects of *Psidium guajava* extract as one mechanism of its antidiarrhoeal action. Malaysian J. Med. Sci., 6: 17-20.

NCCLS, 1997a. Performance Standards for Antimicrobial Disk Susceptibility Test: Approved Standard M2-A6. 6th Edn., National Committee for Clinical Laboratory Standards, Wayne, PA., USA.

NCCLS, 1997b. Reference Method for Broth Dilution Antifungal Susceptibility of Yeasts: Approved Standards M27-A2. 2nd Edn., National Committee for Clinical Laboratory Standards, Wayne, PA., USA.

Ndukwe, I.G., A.I. Bello, J.D. Habila and C. John, 2007. Phytochemical and antimicrobial screening of the crude petroleum spirit and methanol extracts of the stem bark, leaves and roots of *Ficus thonigii* (blume). Afr. J. Biotechnol., 6: 2645-2649.

Saboo, S.S., G.G. Tapadiya and S.S. Khadabadi, 2013. Antimicrobial potential of tropical plant *Trichodesma indicum* and *Trichodesma sedgwickianum*. Res. J. Microbiol., 8: 63-69.

Shivalingappa, H., J. Birader and K. Srudresh, 1999. Antiimplantation activity of alcoholic extract of *Rivea hypocrateriformis*. Indian J. Pharm. Sci., 61: 1309-1310.