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Comparative study on phytochemical, antimicrobial and antioxidant activity of *Sapindus mukorossi* Gaertn. and *Rheum emodi* Wall. ex Meissn.: *In vitro* studies

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Abstract

The work designed to investigate the phytochemical evaluation, *in vitro* antimicrobial and antioxidant activity of *Sapindus mukorossi* Gaertn. and *Rheum emodi* Wall. ex Meissn. The preliminary Phytochemical analysis demonstrated the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins, amino acids and lipids/fats simultaneously. Thin layer chromatography (TLC) also performed and confirmed based on the Rf values reported in the literature. Antimicrobial activity carried out for *Bacillus subtilis*, *Bacillus cereus*, *Proteus valgaris, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia* by agar diffusion method, using ciprofloxacin as standard, respectively. Both the extracts showed a broad spectrum antimicrobial activity by inhibiting the growth of test microorganisms. At the concentration of 10 µg/ml *Rheum emodi* and 10 µg/ml *S. mukorossi* extracts were showed significant rate of inhibition against all microorganisms. The antioxidant activity of the plant extracts were determined by DPPH assay method, using rutin as standard, respectively. IC_{50} values were also calculated. In conclusion, the results indicate that, the *S. mukorossi* and *R. emodi* can be used as a potential source of natural antimicrobial compound possessing strong antioxidant potential.

Key words: *Sapindus mukorossi* Gaertn., *Rheum emodi* Wall. ex Meissn. antioxidants, antimicrobial agents, *in vitro*, TLC DPPH

1. Introduction

Medicinal plants contain substances that can be used for therapeutic purposes, which are precursors for chemopharmaceutical semisynthesis. When a plant is designated as medicinal, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may, therefore, be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents and are used for medicinal purposes. The World Health Organization (WHO) is encouraging, promoting and facilitating the effective use of herbal medicine in developing countries as approximately 90% of the world inhabitants rely on traditional medicine for their primary healthcare needs (Patwardhan, 2005).

A number of medicinal plants are used in traditional system of medicine for the management of liver disorders. Nature has given us a large number of medicinal plants, some of which are yet to be explored and validated for their medicinal value. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver diseases, carefully synergizing the strengths of traditional medicine with the modern concept of evidence based medical evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy. Several herbs are known to possess antioxidant properties and may be useful as liver protective agents (Awen *et al*., 2010). Plant saponins and anthraquinones are widely distributed amongst plants and have a wide range of biological properties. The more recent investigations and findings on plant saponins and anthraquinones have reported many biological activities (Francis *et al*., 2002). In view of these reports, plants containing saponins and anthraquinones were selected to know the antioxidant and antimicrobial potential of *S. mukorossi and R. emodi.*

The genus *Sapindus* belongs to the family Sapindaceae, which has about 2000 species (Watson and Dallwitz, 2007). Most of the species of the *Sapindus* genus are in use for the treatment of several diseases and other commercial purposes. *S. mukorossi* commonly known as a Reetha, is a deciduous tree. The tree is indigenous to northern and central India and is widely distributed in the Himalayan region, Haryana, Uttar Pradesh, and Chhattisgarh (Bauer *et al*., 1966; Ibrahim *et al*, 2008). Traditionally, it is used in the treatment of asthma, snakebite, tooth disorders, piles, dermatological disorders and hepatic disorders. It is a rich source of potential biological activities (Chirva *et al*., 1969, 1970; De Silva, 1997; Huang *et al*., 2005; Tiwari and Singh, 2008). A survey of the chemical literature reveals that a great deal of phytochemical work on different parts

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such as fruit, pericarp, seeds, leaves, ripe fruit, roots, and stems of *S. mukorossi, S. saponaira, S. trifoliatus, etc*., has been carried out by Ibrahim *et al*. (2006).

R. emodi is an important medicinal plant which is extensively used in Ayureda and Unani systems of medicine. *R. emodi* is commonly known as Indian Rhubarb/ Revanchini, belongs to family Polygonaceae, is a leafy perennial herb, distributed in altitudes ranging from 2800 to 3800 m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India (Ibrahim *et al*., 2006). It has the property of purgative, hemostatic, antipyretic, antihelmintic, laxative, atonic indigestion, constipation, diarrhea, dysentery, antibacterial, antitumor, diuretic, hemostatic, cholagogue, antihypertensive and lowers serum cholesterol (Oshio and Kawamura, 1985; Zhou and Chen, 1988; Lu and Chen, 1989; Marian Valko *et al*., 2007; Nautiyal *et al*., 2003).

This current study aimed to assess the phytocompounds by TLC, *in vitro* antimicrobial and antioxidant activity of *S. mukorossi* and *R. emodi.*

2. Materials and Methods

2.1 Collection and authentication of the plant material

The dried pericarp of *S. mukorossi* and the dried rhizome of *R. emodi* were collected from herbal museum of Nizam Institute of Pharmacy, Deshmukhi, Nalgonda, India.

It was recognized and authenticated by Botany Department, Faculty of Science, Osmania University, Hyderabad-500007, Telangana State, India (Voucher No: OU 1028).

2.2 Preparation of extracts

One kilogram (1 kg) of shade dried rhizomes of *R. emodi* powder was extracted successively with hydroalcohol (ethanol and water in a ratio of 70:30) in Soxhlet Extractor for 48 h at 60°C. After extraction, the solvent was evaporated to dryness at 40°C by using a rotary evaporator. The yield was 8 g/kg and was stored at 4°C.

One kilogram (1 kg) of defatted air-dried pericarp of *S. mukorossi* powder, was extracted successively with hydroalcohol (ethanol and water in a ratio of 70:30) in Soxhlet Apparatus, set at 60°C for 48 h. The solvent was evaporated at 50°C, using rotary vacuum evaporator. The yield was 5 g/kg and was stored at 4°C.

2.3 Phytochemical analysis

Phytochemical screening of *R. emodi* and *S. mukorossi* extracts were carried out, using the extract for different types of chemical constituents as per the method described by Trease and Evans (1985). The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids and lipids/fats.

2.4 Identification of functional components using TLC

Thin Layer Chromatography (TLC) is a fast and inexpensive form of chromatography that has many uses in the organic laboratory. Amongst these are: (i) Identify the components in a mixture, (ii) Monitor a chemical reaction, (iii) Identify the proper conditions for a column chromatographic separation and (iv) Purify a sample as part of a preparative procedure. Silica gel coated TLC plates were purchased and used for the study. A line was drawn on the TLC plate at a distance 2 cm from the base, marks were made on the line for sample application. The sample was spotted on the line with the help of capillary tube and it was allowed to dry. The plate was placed in the developing jar with mobile phase. After the solvent reaches $\frac{3}{4}$ th of the TLC plate, it is taken out of the jar, the solvent front was drawn. The plates were then kept in iodine jar for few seconds, shaken and taken out. They were examined under the UV/ Vis lamp and the spots were circled with pencil. The spots were labeled and the distances from the base lines were measured. The Rf values were calculated by the following formula :

$$\text{Retention factor Rf} = \frac{\text{Distance travelled by solute from origin}}{\text{Distance travelled by solvent from origin}}$$

S. mukorossi extract: TLC profile using precoated silica gel plates as stationary phase, Ethyl acetate: Methanol: Water (81:11:8) and anisaldehyde-Sulphuric acid as spray reagent. *R. emodi* extract: Thin layer chromatography (TLC) profile using precoated silica gel plates (60 F254), the stationary phase, Petroleum ether: Ethyl acetate: Formic acid (75:25: 1), and 10% methnolic KOH as the spray reagent.

2.5 Antimicrobial property of *S. mukorossi* and *R. emodi*

The antimicrobial activity of the *R. emodi* and *S. mukorossi* extracts were tested individually on six different microorganisms: *E. coli* (MTCC: 41), *P. aeruginosa* (MTCC: 424), *K. pneumonia* (MTCC: 39), *B. cereus* (MTCC: 430), *P. valgaris* (MTCC: 426) and *B. subtilis* (MTCC: 441). All bacterial strains were obtained from the Department of Microbiology, Nizam Medical College, Hyderabad, India. All the test strains were maintained on nutrient agar slope (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at room temperature and were sub-cultured into newly prepared nutrient agar slants, every two-week. It was investigated by using stokes disc diffusion sensitivity technique and well diffusion methods (Oshio and Kawamura, 1985). In stokes disc diffusion method, a loop of bacteria from the agar slant stock was cultured in nutrient broth over night and spread with a sterile cotton swap into petriplates, containing 10 ml of nutrient agar medium. Sterile filter paper discs (9 mm in diameter) impregnated with the plant extract, were placed on the cultured plates and incubated at 37°C for 24 h. The solvent without extracts served as negative control. Standard antibiotic streptomycin (10 µg), ampicillin (10 µg), tetracycline (10 µg) and ciprofloxacillin (10 µg) were employed as positive control. After 24 h. of incubation and antibacterial activity was assessed by measuring the inhibition zone. The diameters of the zones of inhibition by the samples were then compared with the diameters of the zones of inhibition produced by the standard antibiotic discs. Each experiment was carried out in triplicate and the mean diameter of the inhibition ones was recorded. Screening of antibacterial activity was performed by well diffusion technique. The nutrient agar plates were seeded with 0.1 ml of standardized inoculums of each of the four test organisms. The inoculums were spread evenly over plate with loop or sterile glass spreader. The inoculated plates were incubated at 37°C for 20 min. After incubation, a standard cork order of 6 mm diameter was used to cut uniform wells on the surface of nutrient agar medium and 10 µl of the extracts was introduced in the well and incubated at 37°C for 24 h. and the one of inhibition was measured in millimeter (mm). Mean zone of inhibition and standard deviations were calculated.

2.6 *In vitro* antioxidant properties of *R. emodi* and *S. mukorossi*

The DPPH radical assay is a suitable model for estimating the total antioxidant potential of antioxidants (Huang *et al*., 2005). The assay was carried out in 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the rutin standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25, 15.625 µg/ml, respectively. The plates were incubated at 37°C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader.

2.7 Statistical analysis

The results were carried out in triplicate. Data were analyzed to calculate the mean and the standard deviation.

3. Results

Phytochemical screening of *R. emodi* and *S. mukorossi* extracts screening was carried out, using the extract for different types of chemical constituents as per the method described by Trease and Evans (1985). The extracts were subjected to preliminary phytochemical investigation for detection of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids and lipids/fats. Presence and absence of different phytoconstituents are presented in Table 1.

Table 1: Phytochemical screening of *R. emodi* and *S. mukorossi* extracts

Test	*R. emodi*	*S. mukorossi*
Alkaloids test	+	-
Carbohydrate test	+	+
Test for sterols and steroids	+	+
Test for cardiac glycoside	-	-
Test for anthraquinone glycoside	+	-
Test for saponins	+	+
Test for flavonoids	+	+
Test for tannins	+	-
Test for fixed oil	-	+
Test for protein and amino acid	+	+
Test for terpenoids	+	+

+ = Presence, - = Absence

The qualitative anthraquinone glycoside by TLC revealed the presence of four spots in *R. emodi* extract, presented in Figure 1A. Compounds with Rf values of 0.52, 0.38, 0.27 and 0.84 confirmed the presence of anthraquinone glycosides. The preliminary phytochemical analysis demonstrated the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins, amino acids and lipids/fats simultaneously. Thin layer chromatography (TLC) also performed and confirmed based on the Rf values reported in the literature (Srinivasarao and Ibrahim, 2013; Sengupta and Basu, 2006; Rai *et al*; 1975).

The antimicrobial activity of *R. emodi* and *S. mukorossi* extracts were tested individually on six different microorganisms. The extracts showed a broad spectrum of antimicrobial activity by inhibiting the growth of test microorganisms. At the concentration of 10 µg/ml, *R. emodi* and *S. mukorossi* extracts showed significant rate of inhibition against all microorganisms compared to the standard antibiotics has been shown in the Table 2 and Figure 1B.

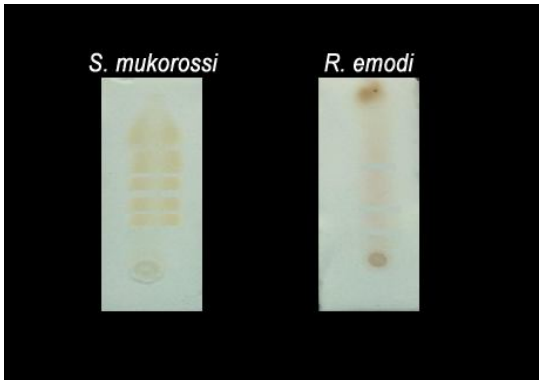

Figure 1A: TLC fingerprint profiles

3.1 Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration where no visible turbidity is observed in the test tube. In this method, the broth dilution technique was used where extracts were prepared to the highest concentration of 1-10 µg / ml(stock solution) by adding sterile distilled water, inoculated with 0.2 ml standard suspension of the test organism after 18-20 h. of incubation at 37°C. The test tubes were observed for turbidity. The minimum inhibitory concentration of test organism was determined, using the tube dilution technique nine milliliter (9 ml) of the nutrient broth was pipettes into various test tubes contains concentration of 10 µg /ml to 1 µg /ml of extract against *Escherichia coli* , *Pseudomonas aeruginosa* , *Klebsiella pneumonia*, *Bacillus cereus* , *Proteus valgaris* and *Bacillus subtilis*. The lowest concentration of the test tube that did not show any visible growth can be considered as the MIC.

Table 2: Antimicrobial property of *R. emodi* and *S. mukorossi*

Microorganism	S.M	R.E	C	T	A	S
Bacillus subtilis	30.8	40.4	30.6	10.5	8.0	20.6
(MTCC: 441)	±0.12	±018	±1.56	±1.82	±0.86	±1.24
Bacillus cereus	20.8	40.8	20.2	20.0	10.0	20.0
(MTCC: 430)	±1.24	±1.02	±0.42	±1.43	±0.46	±1.78
Proteus valgaris	30.0	30.6	40.0	20.7	10.4	30.0
(MTCC: 426)	±0.15	±1.64	±0.68	±1.24	±0.74	±0.17
Pseudomonas aeruginosa	20.8	40.5	30.9	20.6	10.2	20.8
(MTCC: 424)	±1.28	±0.34	±0.72	±0.96	±1.62	±0.69
Escherichia coli	30.0	50.4	40.0	20.6	8.0	8.0
(MTCC: 41)	±0.64	±0.44	±1.24	±0.78	±1.91	±1.29
Klebsiella pneumonia	30.8	50.4	30.4	10.8	-	20.4
(MTCC: 39)	±0.42	±1.42	±1.66	±1.29	-	±1.69

C = chlorfloxacilin, T = tetracycline, A = ampicillin, S = streptomycin, S.M = *S. mukorossi*, R.E = *R. emodi*

3.1 *In vitro* antioxidant activities of *R. emodi* and *S. mukorossi*

DPPH radical scavenging activity has been widely used to evaluate the antioxidant activity of plant extracts and foods. The presence of antioxidant in the sample extract react with DPPH, which is a stable free radical, and convert it to 1,1 diphenyl 2 (2,4,6 trinitrophenyl) hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant compounds which can be detected spectrophotometrically at 517 nm. Figure 1C shows the DPPH radicals scavenging capacity of extracts *R. emodi* and *S. mukorossi* with reference to rutin. Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was calculated. Therefore, lower value indicates a higher antioxidant activity. The experimental data indicated that extracts of *R.emodi* and *S. mukorossi* displayed the highest DPPH scavenging effect 96 (Tables 3 and 4).

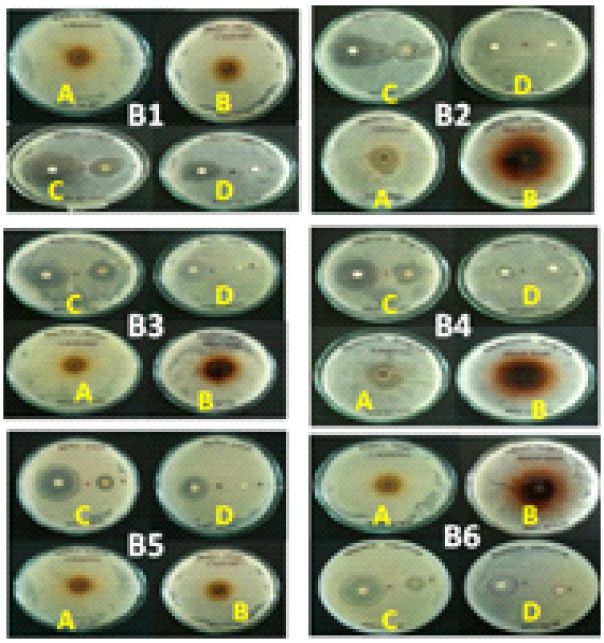

Figure 1B : (Slide B1: *Bacillus subtilis*, Slide B2: *Bacillus cereus*, Slide B3: *Proteus valgaris*, Slide B4: *Pseudomonas aeruginosa* , Slide B5: *Escherichia coli*, Slide B6: *Klebsiella pneumonia*) (A: *R. emodi*, B : *S. mukorossi*, C: *Chlorfloxacilin, Tetracycline*, D: *Ampicillin, Streptomycin*)

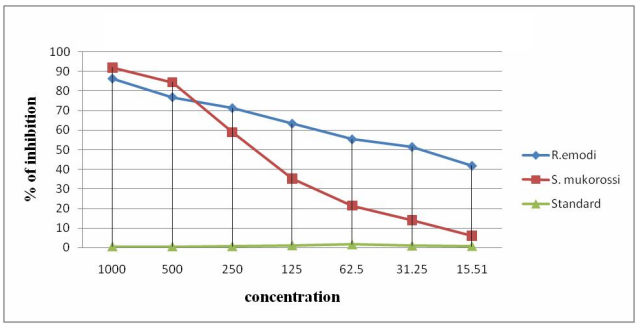

Figure 1C : Antioxidant activities of *R. emodi* and *S. mukorossi*

Table 3: Free radical scavenging activity of *R. emodi* and *S. mukorossi*

Samples	DPPH	
Extract	*R.emodi*	*S. mukorossi*
	30.33±0.0.58	29.33±5.77
Standard	Rutin	
	3.91 ± 0.10	

Table 4 : DPPH radical scavenging activity of *R. emodi* and *S. mukorossi*

conc.	Avg % Inhibition		SD
	R.emodi	*S. mukorossi*	
1000	86.33	91.97	0.59
500	76.88	84.46	0.54
250	71.30	58.96	0.70
125	63.41	35.19	1.12
62.5	55.38	21.45	1.61
31.25	51.49	14.13	0.96
15.51	41.90	6.03	0.74

4. Discussion

This study on the pharmacognostical and phytochemical analysis of *R. emodi* and *S. mukorossi* revealed a set of parameters which may enable to those who handle these plants to maintain its quality control. Adulteration and substitution have become a major problem due to the absence of standards relating to genuineness of drug. Skill hand and cost factors for pharmaceuticals purposes, the quality of medicine must be as high as that of other medicinal preparations. Quality refers to intrinsic value of the drug, the amount of medicinal principles or active constituents present. The pharmacognostical parameters including TLC are helpful for the future identification and authentification of these plants in the herbal industry. The physical parameters, such as loss on drying, ash values and extractive values will be helpful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing these plants in future. Using these standards, the plant can be differentiated from other related species. The plants may be considered as biosynthetic laboratory for a variety of compounds (secondary metabolites) like alkaloids, glycosides, flavonoids, volatile oils and saponin that exert physiological effects. The curative properties of medicinal plants are due to the presence of various secondary metabolites. Thus, the preliminary screening tests may be useful in the detection of bioactive principles. TLC results indicate the number of constituents and further facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Separation and identification were performed for this study only with TLC. Phytochemical study was also useful to isolate the pharmacologically active principles, present in the drug. More phytochemical research work is required for isolation, purification and characterization of biologically compounds. The *R. emodi* and *S. mukorossi* have been used for thousands of years for its medicinal properties. It is rich in a wide variety of secondary metabolites such as glycosides, alkaloids, phytosterols, proteins, saponins and

phytosterols which have been found *in vitro* to have *H. pylori* properties (Ibrahim *et al.*, 2006). In this connection, the present study on the *R. emodi* and *S. mukorossi* extracts was conducted to evaluate the antimicrobial activity. *R. emodi* and *S. mukorossi* showed significant antimicrobial activity compared to standard antibiotics. Preliminary results of the activity of antimicrobial agents such as plant active components, antibiotics are usually expressed *in vitro* as zones of inhibition around the chemical. This is in comparable to the work of (Gislene *et al.*, 2000) on the antibacterial activity of the plant extract and phytochemicals on antibiotic resistance bacteria. According to them, any chemical that demonstrates activity with zones of inhibition of 5 mm and above is acceptable as being active. The extracts of *R. emodi* and *S. mukorossi* showed 8 mm inhibition zone, therefore, it contains effective antimicrobial compounds. *R. emodi* and *S. mukorossi* extracts showed broad spectrum antimicrobial activity.

The screening of antioxidants derived from natural sources has gained much attention and efforts have been put into identifying compounds as suitable antioxidants to replace synthetic ones. Natural extracts with proven antioxidant activity are usually composed with their phenolic moiety, for example anthraquinone glycosides, flavonoids and saponins. The search for phytochemicals with potent antioxidant continues to be of great importance in the search for remedies against free radical-mediated diseases and possible substances with wide range of pharmacological activities such as anti-bacterial and antifungal properties.

5. Conclusion

From our study and with previous literature survey clearly indicates that *R. emodi* and *S. mukorossi* are rich in phytochemicals which have potent antimicrobial activity against selected microorganisms. This study showed that *R. emodi* and *S. mukorossi* possesses significant antioxidant activity. Owing to these properties, this plant has the potential as natural source of antioxidants, capable of protecting against free radical mediated damage and may have applications in preventing and curing various diseases. Further studies require the isolation and characterization of the antioxidant substances and their potential.

Conflict of interest

We declare that we have no conflict of interest.

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