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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF TWO COMMON TERRESTRIAL MEDICINAL PLANTS *Ruta chalepensis & Rumex nervosus*

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Abstract

Ruta chalapensis and *Rumex nervosus* are used as an extensive household remedy for various diseases in Eritrea. The components of these plants are of great interest in medicinal chemistry. Leaves and young stems of *Ruta* and *Rumex* have been reported to contain alkaloids, flavonoids, phenols, amino acids, furanocoumarins and saponins. Various solvents like ethanol, acetone and aqueous extracts of the two plants were screened for the presence of bioactive compounds. The antibacterial activities of these extracts were investigated against *Staphylococcus aureus*, a gram positive bacteria and *Escherichia coli*,a gram negative bacteria. The antibacterial activity was tested using Muller Hinton Agar medium by disc diffusion method and minimum inhibitory concentration assays. After incubation, zone of inhibition was measured in mm, a good inhibition (>5mm) was observed indicating the effective antibacterial activity of the bioactive compounds in both the plant extracts.

INTRODUCTION

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs. Medicinal plants are the sources of bioactive compounds used mainly for medicinal purposes.In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, the undesirable side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobials[9]. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant tannins have anti bacterial activity[18]. Reports also show that flavonoids have anti viral[16] and anti microbial[15] activity and alkaloids extracted from plants commonly have anti microbial property[1]. In this study two commonly used Eritrean traditional medicines Chena adam (*Ruta chalpensis*) and Hihot (*Rumex nervosus*) will be studied for the phytochemical screening and anti bacterial activity.

Rue (*Ruta chalpensis*) is a genus of strongly scented evergreen sub shrubs 20–60 cm tall, in the family Rutaceae, distributed in temperate and tropical countries and was introduced to America after the Spanish conquest. The genus name "Ruta" comes from the Greek word "reuo ", to set free, showing its reputation as a free from disease. There are perhaps 8 to 40 species in the genus. A well-known species is the *Rue*. The leaves are bipinnate or tripinnate, with a feathery appearance, and green to strongly glaucous blue-green in colour. The flowers are yellow, with 4–5 petals, about 1 cm diameter, and borne in cymes. The fruit is a 4-5 lobed capsule, containing numerous seeds[10].

The leaves and young stems have been reported to contain alkaloids, flavonoids, phenols, aminoacids, furanocoumarins and saponins. In addition, the phytochemical screening of the aerial parts of *R. chalepensis* was conducted for the determination of alkaloids, cardiac glycosides, flavonoids, tannins, coumarins, anthraquinones, saponins, volatile oil, volatile bases, cynagenic glycosides, glucosinolates, sterols and/or triterpenes[7]. *Ruta* is also one of the most frequently used plants for medicinal purposes. The characteristic odour of the plant and volatile oil is due to methyl n-nonyl ketone[19]. There are two main species of *Ruta* used in traditional medicine; *Ruta chalepensis* and *Ruta graveolens*[14]. Traditionally, *Ruta* is also used as remedy for many inflammatory diseases[6]. Furthermore, extracts from rue have been used to treat eyestrain, sore eyes, and as insect repellent. *Rue* has been used internally as an antispasmodic, as a treatment for menstrual problems, as an abortifacient, and as a sedative.In Saudi Arabia, a decoction of the aerial parts of the plant is used as an analgesic and antipyretic and for the treatment of rheumatism and mental disorders. The plant is prescribed in the Indian system of medicine for the treatment of dropsy, neuralgia, rheumatism and menstrual and other bleeding disorders. In China, a decoction of the roots of the plant is used as anti-venom. The extracts of *Ruta* have an oxidative property which can control the colon cancer[4]. During the tropical usage of the *Rue* extracts care must be taken, after applying to the skin with sun exposure, the oil and leaves can cause blistering. Traditionally, in Eritrea, the leaves of *Ruta* are used for myalgia, cold, whooping cough, abdominal pain, anti-emetic and many more.

Fig1: The leaves and flowers of *R.chalepenis*

Fig2: Effect of *R.chalepensis* in hot weather

*Rumex nervosus*is commonly found near and around the terraces of high altitude areas (above 1000m.). Genus *Rumex* is a genus of about 200 species of annual, biennial and perennial herbs in the buckwheat family Polygonaceae. Members of this family are very common perennial herbs growing mainly in the northern hemisphere, but various species have been introduced almost everywhere. *Rumex nervous* leaves are an edible, consumed by some people in Saudi Arabia. In Eritrea the leaves and stem of this herb is used for traditional medicine by the practitioners mostly on highland and on the villages it is used for purifying the body by women (traditionally known *'tish'*) as substituent of olive tree, to do this, the leaves are put on fire then they cover the patient body with that hot leaves and blanket so that the vapours and smoke surround all the body.

Rumex species are used as food plants by the larvae of a number of Lepidoptera species. The leaves of most species contain oxalic acid and tannin, and many have as tringent and slightly purgative qualities. Some species with particularly high levels of oxalic acid are called *sorrels* (including sheep's sorrel, *Rumex acetosella*, common sorrel, *Rumex acetosa* and French sorrel, *Rumex scutatus*), and some of these are grown as pot herbs or garden herbs for their acidic taste. *Rumex* species contains anthracene derivatives like chrysophanol, physcion, emodin, aloe-emodin, rhein; which are the main biologically active compounds responsible for anti-cancer, cytotoxic, genotoxic and mutagenicity properties[20]. Traditionally in Eritrea, the leaves, stems and sometimes roots of *Rumex nervosus* are used as traditional medicines, for the eye disease, taeniacapitis, haemorrhoids, infected wounds, arthritis, eczema, abscess and gynecological disorders.

Fig 3: The leaves and flower *Rumex nervosus*

MATERIALS AND METHODS:

Collection of plant material:

The leaves of juvenile plants of *Ruta chalepensis* and *Rumex nervosus* were collected from the central region surroundings of Asmara at an altitude of 2300 meters, Eritrea and were authorized by department of botany, Eritrean Institute of Technologyand the specimen voucher was preserved in the herbarium.

Extraction procedure:

The plant material was dried in shade, pulverized in household mixture. The powdered material was successively extracted by maceration in various solvents with different polarities like acetone, water and ethanol for four weeks. All the extracts were concentrated by evaporating the solvents, the concentrated extracts were used for phytochemical screening and antimicrobial activity[2,8,21].

Microbial strains:

The bacterial strains of *Staphylococcus aureus* and *Escherichia col*i were procured from the quality control laboratory, Massawa, Eritrea.

Phytochemical screening:

The extracts of the dry powdered leaves of *Ruta chalpensis and Rumex nervosus* were analyzed for the presence of various phytochemical constituents likecarbohydrates, reducing sugars, monosaccharide, Tannins, Saponnins, Flavonoids, Terpenes/steroids (Liebermann - Burchard's Test), Alkaloids, Anthraquinones (Borntrager's test), cardiac glucosides (sodium nitroproside method) proteins (copper sulphate and FolinCiocalteausolution) and amino acids(Ninhydrin) wereidentified using standard phytochemicalprocedures[8, 11,12,17]. The results were shown in table 1.

Anti microbial activity:

The various solvent leaves extracts of *Ruta chalpensis* (Chena adam) and *Rumex nervosus* (Hihot) were tested by the disc diffusion method[3]. The test microorganisms were seeded into Muller Hinton Agar medium by swab method of 10 µl(10 cells/ml)

with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. *E. coli* and *S. aureus* were used for antibacterial test. Tetracycline positive control and solvent of the extract was used as a negative (or reference) control .The antibacterial assay plates were then incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm. Diameters less than 5 mm indicate no effect.

RESULTS AND DISCUSSION:

Phytochemical Screening:

The results of the phytochemical analysis investigated for *Ruta chalpensis* and *Rumex nervosus* using in different solvent (acetone, ethanol, and aqueous) extracts are presented in Table 1. Both plants showed the presence of different types of active constituents like alkaloids, flavonoids, terpenoids, tannins, glycosides, volatile oils, etc... [2]

Majority of the bioactive components were extracted in the acetone and ethanol extracts. Only Flavonoids, tannines and cardiac glycosides, secondary metabolites, are extracted on the aqueous extraction. Saponines are present in leaves and young stems of the *Ruta chalpensis* plant[5]. These saponines are one of the active components having anti microbial activity[22] .

Antimicrobial Assay activity:

The results of antibacterial activity of *R. chalpensis* and *R. nervosus* leaves extracts are shown in table 2 and Table 3. All extracts showed activity. The acetone extract of *R. chalpensis* showed maximum growth inhibition (8.5 mm) against gram negative bacteria. While ethanol extract of the same plant showed maximum growth inhibition against gram positive bacteria (8 mm).So the plant showed significant inhibition for gram positive and gram negative bacteria. More over, *R. chalpensis* has good inhibition for gram positive bacteria in all the extracts (Fig 4 and 5).

Results also showed that *R. nervosus* has inhibitory effect for gram positive bacteria only in the ethanol extract. But it has more inhibition against Gram negative bacteria in the ethanol and aqueous extracts. The plant showed maximum inhibition of (6.5 mm).

The activities observed for both plants are in concordant with the positive control that inhibited with an average of 9mm in both the positive and negative gram bacteria. *E. coli* and *S. aureus* which are also resistant to different antibiotics had their growth inhibited by acetone, ethanol and aqueous extracts of *R. chalpensis* and *R. nervosus*. (Fig 6 and 7)

Table 1. Phytochemical screening of the leaves of *Ruta chalpensis* and *Rumex nervosus*

NO	*Chemical Tests*		SOLVENTS					
			Acetone		Ethanol		Water	
			Rumex	*Ruta*	*Rumex*	*Ruta*	*Rumex*	*Ruta*
1	Test for Carbohydrates							
	1	Molish's test	+	+	+	+	+	+
2	Tests for Reducing sugars							
	1	Fehling's test	+	+	+	+	+	+
3	Test for Monosaccharides							
	1	Barfoed's test	+	+	+	+	+	+
4	Tests for Fats & Oils							
	1	Solubility test	+	+	+	+	-	-
5	Test for Proteins							
	1	Xanthoproteic test	+	+	+	+	+	+
6	Tests for Alkaloids							
	1	Mayer's test	-	-	+	+	-	-
7	Tests for Flavonoids							
	1	Conc. H_2SO_4 test	+	+	+	+	+	+
8	Test for Terpenes/Steroids							

	1	Liebermann-Burchard test	+	+	+	+	-	-
9	Test for Glycosides							
	1	Cardiac glycosides						
		a. Keller-Killani test	+	+	+	+	+	+
	2	Anthraquinone glycosides						
		a. Borntrager's test	+	+	-	-	-	-
	3	Saponin glycoside						
		a. Foam test	+	+	+	+	-	+
10	Test for Tannins /Phenolic compounds							
	1	Test with $FeCl_3$ solution	+	+	+	+	+	+

Table 2. Measurement of inhibition zones of *Ruta chalpensis*

Test organism	Average Diameter of inhibition zone(mm) for Rutaceae Extracts			
	Standard	Ethanol extract	Acetone extract	Water extract
E. coli	9	7.5	8.5	0
S.aureus	9	8	6.5	5

Table 3. Measurement of inhibition zones of *Rumex nervosus*

Test organism	Average Diameter of inhibition zone(mm) for Rumex Extracts			
	Standard	Ethanol Extract	Acetone Extract	Water Extract
E. coli	9	0	6	6.5
S.aureus	9	5	0	0

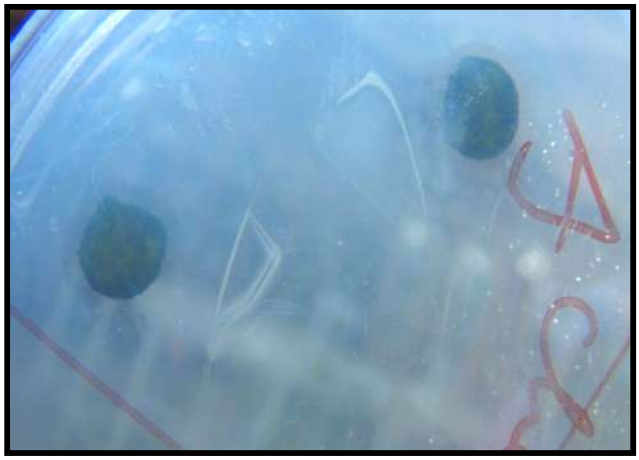

Fig. 4: Antimicrobial activity of Acetonic extract of *Rutaceae* (Chena Adam) against *E. Coli*

Fig. 5: Antimicrobial activity of Ethanolic extract of *Rutaceae* (Chena Adam) against *E. Coli*

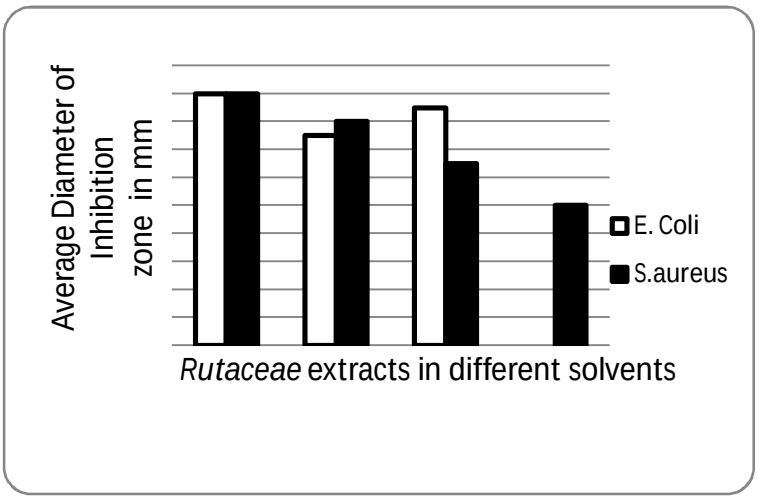

Fig. 6. Antibacterial activity of *Ruta chalpensis* against gram positive and gram negative bacteria

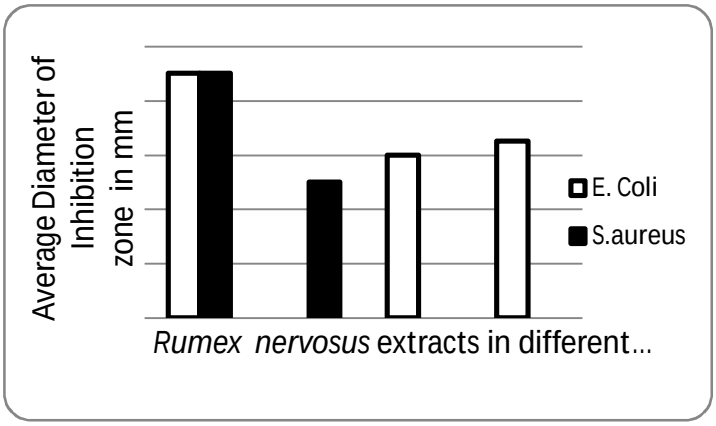

Fig. 7.Antibacterial activity of *Rumex nervosus* against gram positive and gram negative bacteria

CONCLUSION:

The two terrestrial medicinal plants *Ruta chalapensis* and *Rumex nervosus* are rich in secondary metabolites like alkaloids, flavonoids, tannins, steroids, cardiac glycosides, etc[2]. The presence of various bioactive compounds justifies the use of whole plant for various diseases by traditional practitioners in Eritrea.Most of the bioactive components were extracted in the acetone and ethanol extracts. So the acetone or ethanol extracts are more essential as traditional medicines. The results suggest that the presence of flavonoids, cardiac glycosides and tannins/phenolic compounds of these plants may contribute to their claimed antibacterial property in aqueous extracts.

Various workers have shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria[5, 13] and in this study it is evident that all extracts of *Rutaceae s*howed significant activity against Gram positive bacteria *S. aureus* which is similar to inhibition zone observed for positive control Tetracycline.

Further ways of extraction and spectroscopic isolation of compounds from these two plants is, however, required to confirm specificity of the compounds responsible for antimicrobial activity.

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