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Effect of various extracts of the leaves of *Borreria hispida* (Linn) on antibacterial activity

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ABSTRACT

The aim of this to evaluate hydroalcohol of antibacterial activity of extracts from leaves of *Borreria hispida*. Phytochemical screening of various extracts such as ethyl acetate, acetone, chloroform, methanol and hytroalcohol of leaves extracts, revealed the presence of saponins, phenols, flavonoids, cardiac glycosides, carbohydrate, tannins, quinones, and alkaloids. Different concentrations of hytro alcoholic leaves extracts were tested for the anti-bacterial activity. This study shows that medicinal plants has been the sources of compounds which can be used to fight against *Bacillus subtilis*, *B.cereus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* using the agar disc diffusion technique. The powerful antibacterial effect is attributed to be high effect of hytroalcohollic leaf extracts (75% methanol and 30%water) compare than other solvent of leaves extract of *Borreria hispida*. The hytro alcoholic extracts from dry powdered leaves of *Borreria hispida* had superior level of antimicrobial activity.

Keywords: *Borreria hispida*, Phytochemical analysis, Disc diffusion, Antibacterial activity and Hydro alcohol.

1. INTRODUCTION

Medicinal plants are rich sources of bioactive agents, includes, antimicrobial, anticancer and antioxidant agents [1-3]. A wide range of medicinal parts are used to get different rasayanas which possess different medicinal properties against different microbes. Although hundred of plants species have been tested for antimicrobial properties, the majority of these have not been adequately evaluated. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [4-6]. Most of the people in rural and urban areas of the world are depended on the medicinal plants for the treatment of infectious diseases. Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Dietary phytochemicals are considered as an effective tool to cure body disorder. They play important roles as therapeutic agents in prevention of many diseases [5]. Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed wide range of antioxidant, antibacterial and antifungal activity [9-10]. Many more compounds have high rich sources of polyphenol present in plants, foods, and beverages, soluble in water and polar organic solvents. These hydroalcoholic leaf extrcat are classified as soxhlet method and its following condensation based on their biological activity [11-12].

Figure - 1: Morphology of *Borreria hispida(Linn)*.

The crude plant extracts activity against all bacteria tested with inhibition zones in the range of 8-24 mm. The minimal inhibitory concentration (MIC) values of different plant extracts against the tested bacteria were found the most active plant extracts were from *Borreria hispida*. In recent years, hytro alcohol IC_{50} value have been investigated to possess high antioxidants [17], free radical scavenging activity [18], antimicrobial [19], gastro protective, and anti-ulcerogenic activities [20]. Moreover, hytro alcohol extract of polyphenol have been investigated as potent inhibitors of lipid peroxidation in heart mitochondria [21] and possess anti-fibrotic effects [22]. Due to these therapeutic properties polyphenol can be used in the treatment of various diseases to improve human health. *Borreria hispida* (Rubiaceae) is being used in various health care systems for the treatment of various disorders including life threatening diseases.

The *Borreria hispida*, commonly known as "GATHIYU OR SHANKHLO" is perennial herb, easily available and grown as a hedge plant along home gardens throughout the India. Ethno botanically, *Borreria hispida* (Rubiaceae) has been used as therapeutic agent in the treatment of various pathological conditions. Now this study were purpose of *borreria hispida* is used as an antimicrobial an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and anntifungal, fungai. They can also be classified according to their function and also used in cardiovascular disorder etc. It is used as fodder and also consumed as vegetable in times of scarcity. Herb is rich in calcium and phosphorous. Extract of leaves is given for haemorrhoids and gall stones, seeds are used as demulcent and used in diarrhea, dysentery.

2. MATERIALS AND METHODS

The healthy plants of *Borreria hispida* were collected from Thanjavur, Tamilnadu, India. The collected leaves were brought to the laboratory and maintained (Azymes, pvt Ltd, at Bengaluru, Karnataka.)

2.1. Preparation of the plant extracts

Extraction of the plant samples was done according to a combination of the methods used by soxhlet method. The shade dried leaves (50g) of *Borreria hispida* were finely powdered with grain and extracted with 150 ml ethyl acetated, acetone, chloroform, methanol and hytro alcohol separately for 24 hours. The sample was then filtered through Whatman No.1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotaevator at 40ºC to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10°C [23-24].

2.2. Phytochemical screening from leaf extracts of *Borreria hispida*

The powdered samples were tested for the presence of various phytochemicals. Plant extracts were taken with five different solvents such as ethyl acetated, acetone, chloroform, methanol and hytro alcohol. The plant extract was subjected for qualitative phytochemical screening for the presence of bioactive compounds terpenoids, alkaloids, glycosides and cardiac glycosides, steroids, quinines, coumarines, phenols, tannins, flavonoids, saponins, anthocyanin and betacyanin by the standard methods [25-26].

2.3. Anti-bacterial activity of leaf extracts of *Borreria hispida*

The hytro alcohol leaves extracts of *Borreriahispida* plant were used for antibacterial study [27-29]. Different concentrations (10mg, 20mg , 30mg and 40mg /ml) of the concentrated hytro alcoholic leaves extract was tested for its antimicrobial activity against pathogenic bacterial strains such as Bacillus subtilis, B.cereus, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli. The bacterial cultures were grown in Lysogeny broth (**LB**) Agar [30].

2.4. Antibacterial activity assays

Antibacterial activity was measured using the standard method of diffusion disc plates on agar [31-32]. For antimicrobial assay, all bacterial strains were grown in Lysogeny broth (**LB**) Agar Broth Medium for 24 hours at 37° C and plated on Lysogeny broth (**LB**)) for agar diffusion experiments. Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. Sterile disc (Hi Media, 6mm in diameter) were placed on the agar medium to load 20µl of different concentration (10 - 30mg /ml) of hytro alcohol leaves extracts of *Borreria hispida* were tested. Inhibition diameters were measured after incubation for 24 hours at 37° C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

3. RESULTS AND DISCUSSION

Preliminary phytochemical analysis of leaf extracts of Borreria hispida collected from namely Thanjavur, Tamilnadu has been shown in tables 1 respectively. In the present study, phytochemical screening was performed with

ethyl acetated, acetone, chloroform, methanol and hytro alcohol of the leaves extracts of *Borreria hispida*. The phytochemical screening of five different extracts studied, showed that the hytro alcoholic extract of leaves of *Borreria hispida* (Thanjavur) were rich in secondary metabolites such as tannins, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, phenol, steroid, coumarins and alkaloids followed by other accessions. Phytochemical constituents such as polyphenol, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defence mechanism against predation by many micro-organisms, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, saponins, steroids, etc [33]. Polyphenol compounds present in many medicinal plants inhibit the growth of many fungi, yeasts, bacteria and viruses [34]. The presence of alkaloids and saponins in leaves extract, the biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities [35]. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and haemolytic activity [36] and traditionally saponins have been extensively used as detergents, as pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents and also have beneficial health effects [37] Plant steroids are known important for their cardiotonic activities and also used in nutrition, herbal medicine and cosmetics. Thus the preliminary screening tests may be useful in the detection of the bioactive principles, leading to drug discovery and development [38]. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

Table - 1: Phytochemical screening from leaf extracts of *Borreria hispida*

Phytochemical Test	Leaf extracts of *Borreria hispida*				
	Ethylacetate	**Acetone**	**Chloroform**	**Methanol**	**Hydroalcohol**
Tannins	+	+	+	+	++
Saponins	-	-	-	+	+
Quinones	+	+	-	+	+
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Flavonoids	+	-	-	+	++
Phenol	+	+	+	++	++
Alkaloids	+	+	-	-	-
Glycosids	-	-	-	+	+
Cardiacglycosids	+	-	+	+	+
Anthocyanin	-	+	-	-	-
Beta cyanin	+	-	-	+	+

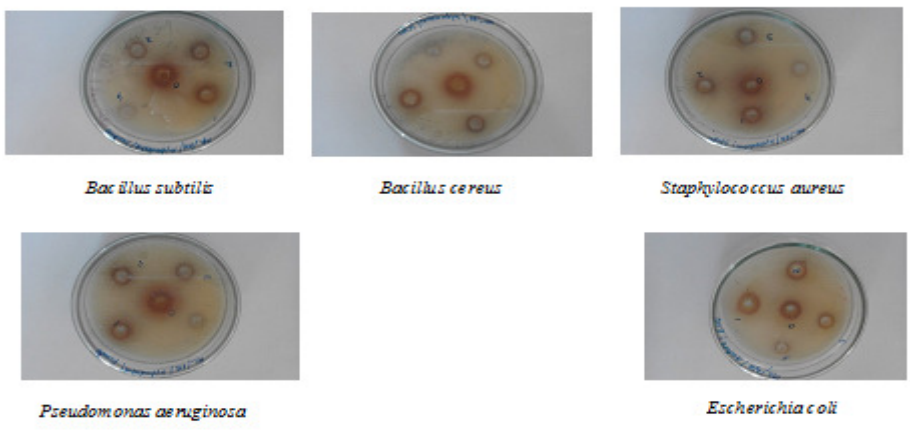

Figure - 2: Antibacterial activity of leaf extract of *Borreria hispida.*

Table - 2: Antibacterial activity of leaf extract of *Borreria hispida*

Name of the pathogens	Zone of inhibition(mm indiameter)* Concentration of extract			
	10mg/ml	20mg/ml	30mg/ml	40mg/ml
*Bacillus subtilis*MTCC No. 10224	11	15	20	24
Bacillus cereus (MTCC 10211)	8	12	16	22
Pseudomonas aeruginosa(MTCC 14676)	8	11	13	17
Staphylococcus aureus(MTCC 9542)	9	13	14	15
Escherichia coli (MTCC 1563)	9	11	12	13

** Includes diameter of disc (6mm); Average of three replicates*

The data presented in table 2, indicate that the leaf extracts of *Borreria hispida* inhibit the growth of some microorganism to various concentration. The concentrations of 10mg/ml–40mg/ml hydroalcoholic extract showed antimicrobial activity against *B. subtilis, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* (Figure 2). The maximum clear zone of inhibition was found at 40mg/ml of 75%hydroalcoholic leaf extract of *Borreria hispida*than other leaf extract**.** In case of leaf extracts, there is no zone of inhibition was found in lower concentration (10mg/ml). Similar results were obtained on antibacterial activity [39-43]. The antimicrobial activities of methanol extract may be due to the presence of tannins, triterpenoids and flavonoids. Thus from ourfindings, it is concluded that the 75% hydroalcoholic extracts from dry powdered leaf of *Borreria hispida* had superior level of antimicrobial activity. The powerful antibacterial effect is the hydroalcoholic leaf extracts of *Borreria hispida*.

4. CONCLUSION

It concludes that the total polyphenol content and antibacterial activity of medicinal plants proved to be very important in identifying new compounds. The present communication assessesment of the status a phytochemicals, total tannin and antibacterial activity in the leaf extract of *Borreria hispida* to improve the health status of the people. It indicates that the plant material become an important source of natural drug compounds, here contained many bioactive chemical constituents including presence of tannins, triterpenoids and flavonoids Phenol, and its requires further testing and through which we could find something ,then we can able to cure any other new disease

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