Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2019; 7(4): 207-211

Received: 21-05-2019
Accepted: 25-06-2019

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Antibacterial and phytochemical evaluation of various extracts of *G. indica* (Kokum) leaves and bark

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Abstract
Objective: To study antibacterial activity of various extracts of leaves and bark of *G. indica* (Kokum) against *E. coli, Klebsiella spp.* and *Salmonella spp.* and their qualitative phytochemical investigation.
Materials and Methods: Six different solvent viz. chloroform, hexane, methanol, ethyl acetate, acetone and aqueous were used for extract preparation. Total 12 extracts were prepared form bark leaves and bark of *G. indica*. Agar cup diffusion method was used to evaluate qualitative antibacterial activity against *E. coli, Klebsiella spp.* and *Salmonella spp.* qualitative phytochemical presence also evaluated by previously described standard protocols with suitable modifications.
Results: All extracts showed considerable antibacterial activity against tested bacterial spp. Hexane, aqueous and acetone extract of leaves was found more antibacterial activity against *E. coli, Klebsiella spp.* and *Salmonella spp.* respectively. Extracts prepared from bark of *G. indica* also showed moderate antibacterial activity against same tested bacterial spp. In that case methanol, chloroform and acetone extracts showed potential antibacterial activity against *E. coli, Klebsiella spp*. and *Salmonella spp.* respectively. Acetone extract of leaves and bark are almost equally effective against *salmonella spp.* it is might be due to the presence of phytochemicals in it. Phytochemicals screening of the both acetone extract showed positive for carbohydrate, flavonoid and tannins in both the acetone extracts, but in both extract acetone extract of bark showed maximum appearance of phytochemicals than the leaves.
Conclusions: The results obtained from present study showed that the *G. indica* is a potential source of various phytochemicals and it can be used in the treatment and prevention of against bacterial infections.

Keywords: G. indica, antibacterial potential, natural medicine

Introduction
Microbial infections have been recognized as a leading cause of immature death in animal and human [1]. On other hand, constant development of superbugs (multi-drug resistant bacteria) which are resistant to almost all the antibacterial compounds available in pharmacy. Therefore screening of new antimicrobial substances from natural resources is a great challenge ever [2]. India have protracted history regarding use of medicinal plant to improve health in animal and human [3]. Medicinal plants becoming increasingly popular in modern society as natural alternatives to chemically synthesized drug molecule because plant derived drugs have been reported to be safe and without side effects [4]. Plant derived metabolites are globally known for its antimicrobial, anti-cancer, anti-diabetic and other bioactive properties. The medicinal plants are easily available in nature and hence it prove that the interrelationship between man and nature [5]. Plant derived medicine are considered to be first line of defense maintaining health and combating diseases and even today plant source is principle source of new drug of therapeutic property [6]. The *Garcinia indica (G. indica)* (Kokum) is most important economical plant mainly found in Western coastal (Maharashtra, Goa, Karnataka) region of India. This plant is belonging to the family *Clusiaceae* [7]. With their economic importance *G. indica* is also popular for their bioactive metabolites which exhibits antimicrobial, anthelminthic and anticancer potential (fig 1) [8]. The leaves and fruits are well known for their sour and astringent taste. The kokum juice and squash made of rind is used to cure various disease such as piles, hemorrhoids, diarrhoea, dysentery, ear infection. The Kokam rinds are commonly used to prepare concentrated syrup also the Kokum butter isolated from the seed is great demand in confectionary medicine and cosmetic industries [8, 9]. Under these circumstances this study is carried out to screen antibacterial and phytochemical properties in different solvent extract of *G. indica* bark and leaves.

Material and method

Collection of plant material

Leaves and bark of *Garcinia indica* were collected during November (winter season) 2014, from the local Kokam plantation located in Golvan, Maharashtra India (latitude16.128394; longitude 73.578349) (fig 2). Samples were collected in plastic bag and transported to Microbiology laboratory of college. Collected specimens were authenticated by plant taxonomy experts.

Processing of plant material

The collected specimens of leaves and bark were cleaned with running tap water and then surface sterilized by using 10% Sodium hypochloride solution. Cleaned specimens were chopped into small pieces and then allowed for drying in the dry shade for five days. Dried specimens were used for fine powder preparation by using mixer grinder. The both powdered samples were stored at cool and dried place separately in two cleaned sterile plastic containers for further study.

Solvent extraction

Extraction of the bioactive components from the powder samples was carried out using Soxhlet apparatus. 20gm of each powder sample were subjected to Soxhlet extraction with six solvents‟ Chloroform, Hexane, Acetone, methanol, ethyl acetate and distilled water. Evaporation of solvent was carried out at 50^{o}C for three days [10] Total twelve extracts were obtained; each extract was transferred into clean and air tight glass tube and stored in cooling condition. Dimethyl sulphoxide (DMSO) was used as diluent to prepare different working stocks from the respective extract.

Antimicrobial activity

The antimicrobial activity of the all extracts was performed according the standard agar- cup diffusion method. 100µl of 6 different concentration of both the extract [100µg, 50, 25, 12.5, 6.25, 3.12 µg/ml] were used to detect qualitative antibacterial activity against *Escherichia coli (E. coli), Klebsiella spp.* and *salmonella spp.* Each assay were carried out in triplicate and DMSO was use as a negative control for entire antibacterial assay.

Qualitative phytochemical analysis

All extract were dissolved in DMSO and processed for phytochemical analysis as per previously described protocol by Desai, *et al*. 2016 [10].

Results and Discussion

Various phytochemicals present in plant materials are responsible for improvement of its medicinal properties and they are helpful to mankind [11]. Screening of plant material for their bioactive potential is an important need of today's era [9, 11]. The antimicrobial activities of higher plants and essential oil derived from them have been well known for centuries. *G. indica* is economically important plant which is already known for their medicinal properties [8]. This study is typically designed to compare antimicrobial potential of various extracts of bark and leaves of *G. indica*. *E.coli, Klebsiella spp.* and *Salmonella spp.* were selected as a representative test organism and antibacterial activity of various extracts of leaf and bark *G. indica* was evaluated against them (Fig 3 and 4). Among the total of six extracts of leaves in this study, Hexane extract of leaves showed maximum antibacterial activity against the tested *E. coli* whereas, acetone extract of the leaves proved to be more potent against *salmonella spp.* than other solvent extracts; but against *Klebsiella spp.* both the extracts showed comparatively less antibacterial potential, in that case aqueous extract of the leaves found to be more potent. Ethyl acetate extract is found to be almost equally effective against all the three tested bacteria. Phytochemical screening of the same extract also showed the ethyl acetate extract contain higher number of phytochemicals (tab 1). The six different extracts prepared from bark of *G. indica* also showed moderate antibacterial activity against same tested bacterial spp. In that case methanol, chloroform and acetone extracts showed potential antibacterial activity against *E. coli, Klebsiella spp*. and *Salmonella spp.* respectively. Chloroform extract of bark showed higher number of phytochemical present. Acetone extract of leaves and bark are almost equally effective against *salmonella spp.* it is might be due to the presence of phytochemicals in it. Phytochemicals screening of the both acetone extract showed positive for carbohydrate, flavonoid and tannins in both the acetone extracts, but in both extract acetone extract of bark showed maximum appearance of phytochemicals than the leaves. Phytochemical screening showed that flavonoid is present in all the extract except aqueous extract of the leaves. Surprisingly concentration of extract interfere in antibacterial activity in some cases lower concentration of extract showed maximum effect than higher concentration of same extract against same tested bacterial spp. it indicates concentration also playing game changing role in screening of natural bioactive compounds. It might be due to because the more concentration may affect speed and amount of diffusion of the extract in culture and due to this affect tested strain got enough time to for survive and grow even in presence of potential extract. Qualitative analysis of phytochemical prove that the plant *G. indica* is potential source of diverse group of phytochemical and it is also already proven that [12, 13, 14]. Total twelve extract were screened for presence of phytochemical out of that carbohydrate and flavonoids is present in total 11 extract except aqueous extract of bark for carbohydrate and leaves extract for flavonoids.

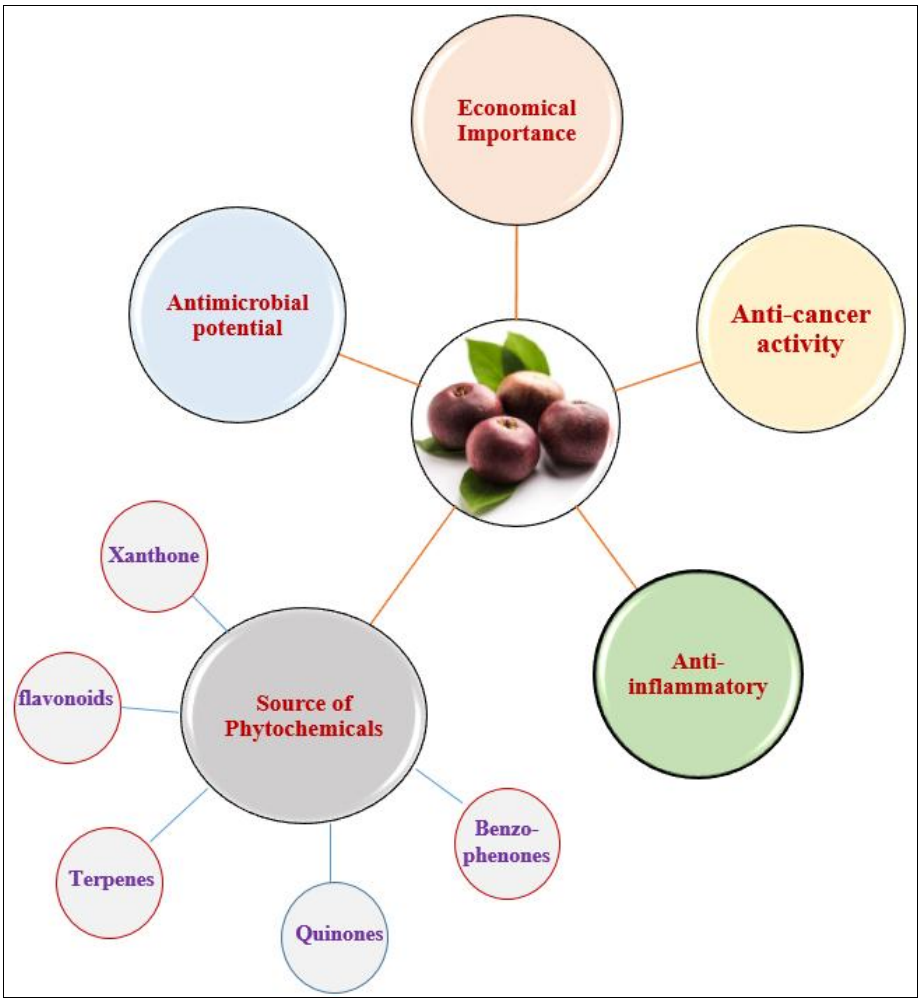

Fig 1: *G. indica*: an important plant

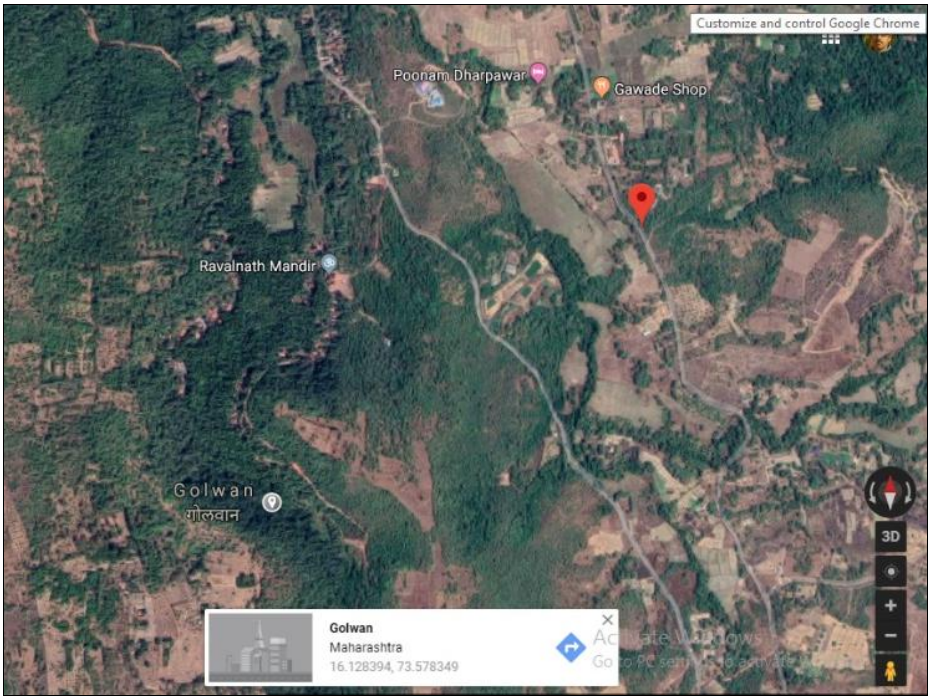

Fig 2: The map shoving sampling region and their geographic coordination

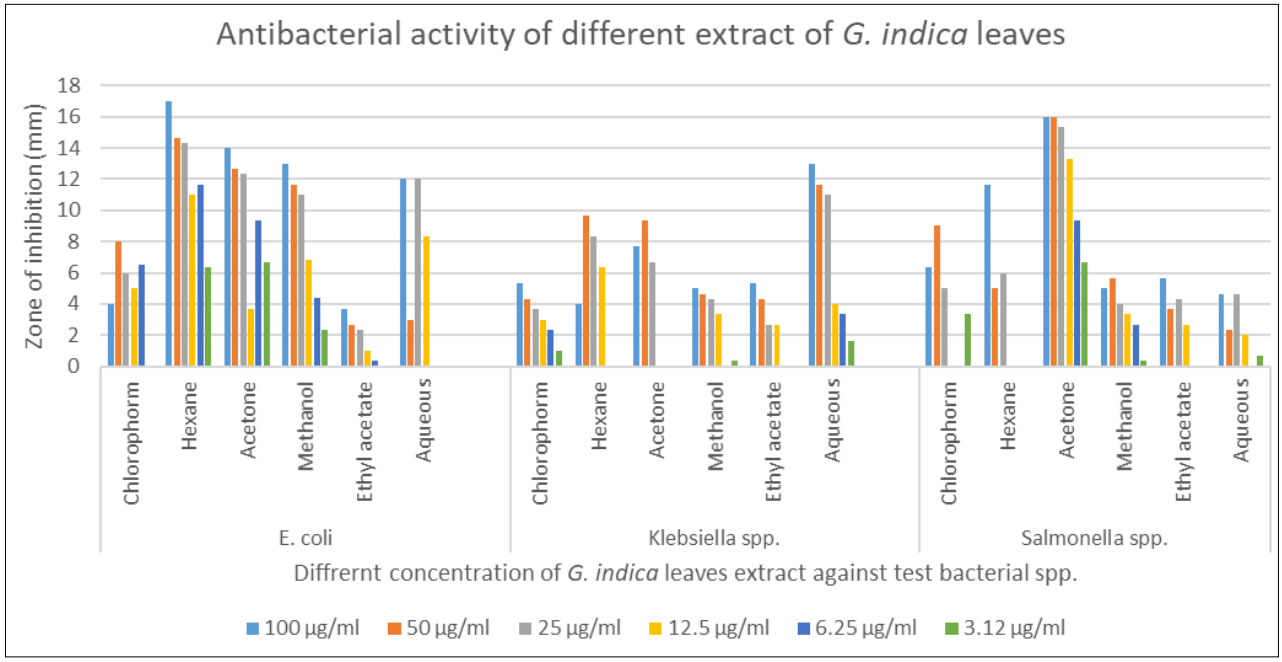

Fig 3: Antibacterial activity of different extract of *G. indica* leaves

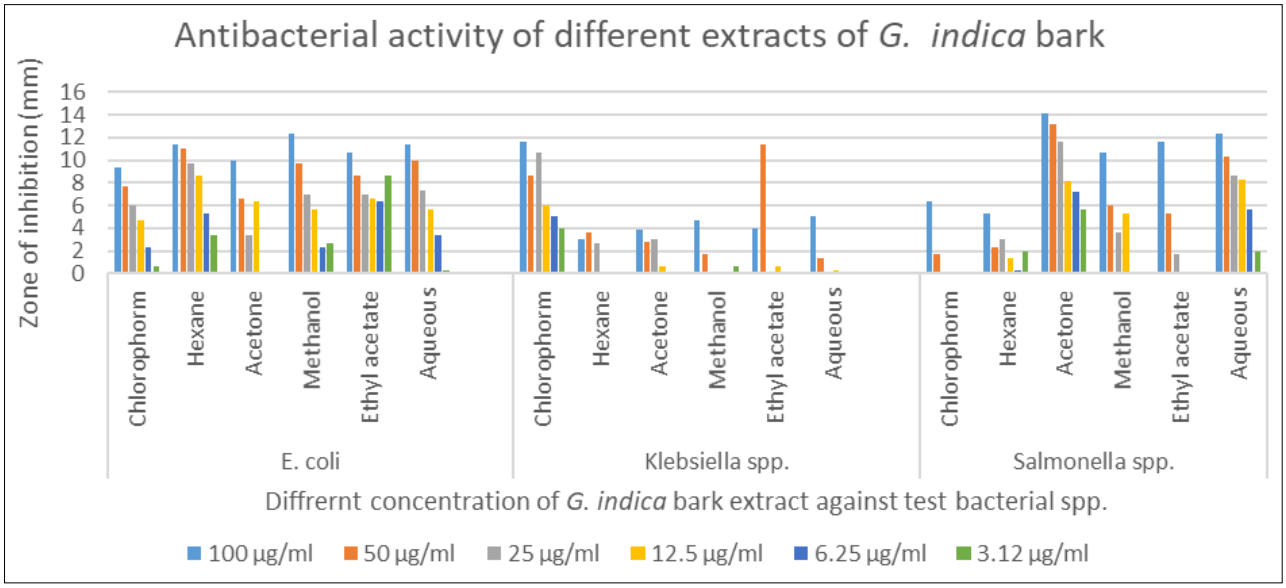

Fig 4: Antibacterial activity of different extract of *G. indica* bark

Table 1: Phytochemical appearance in different extracts of leaves and bark of *G. indica*

Phytochemicals	Leaves extracts						Bark extracts					
	Ex. 01	Ex. 02	Ex. 03	Ex. 04	Ex. 05	Ex. 06	Ex. 01	Ex. 02	Ex. 03	Ex. 04	Ex. 05	Ex. 06
Terpenoids	+	-	-	+	+	-	-	-	+	+	+	-
Alkaloids	-	-	+	+	-	-	+	+	-	+	-	+
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	-
Flavonoids	+	+	+	+	+	-	+	+	+	+	+	+
Phenols	+	+	+	+	-	-	-	-	-	-	-	-
Sterols	-	-	-	-	+	+	+	-	+	+	+	+
Proteins	-	-		-	+	-	+	-	+	+	+	+
Saponins	+	-	+	+	+	+	-	+	-	-	+	+
C. glycosides	+	+	-	+	+	-	+	+	+	+	+	-
Tannins	-	+	+	-	+	-	+	+	+	+	-	+
Oxalate	+	-	-	-	+	+	+	-	+	+	-	+
Quinones	-	+	-	-	-	+	+	+	-	-	+	-
Phlobatannins	-	+	+	+	+	+	-	+	-	-	-	+

Ex. 01 – Chloroform, Ex. 02 – Hexane, Ex. 03 – Acetone, Ex. 04 – Methanol, Ex. 05 - Ethyl acetate, Ex. 06 – Aqueous (+)-Present (-) – Absent

Conclusion

Searching new source of bioactive component is an urgent need. Some plants are already reported effective against pathogenic bacterial infection, therefore the study was carried out to find the herbal remedies. *G. indica* possess number of phytochemicals and this study indicates *G. indica* is a potential source of antibacterial compounds. The extracts possessing high antibacterial effects should be further studied for their detailed analysis and therapeutic use as these plants are easily available, agriculturally important, economically affordable and having medicinal values. Hence, these plants can be used to minimize for achieving a healthy life in human as well as animals.

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