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Estimation of Phytochemical, Antimicrobial and Molecular Comparison of Fruit Extracts of *Garcinia cambogia* and *Tamarindus indica.*

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ABSTRACT

India holds a rich biodiversity of the medicinal plants that were still not exploredcompletely.The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries. The present study was designed to evaluate the phytochemical, antibacterial properties and of tamarind and garcinia fruit extracts. The molecular comparison of *Tamarindus indica* and *Garcinia cambogia* fresh leaves were used for isolation of DNA molecules. The dried fruits of *Tamarindus indica* and *Garcinia cambogia* were collected, dried, and extracted using water, ethanol and hexane. Phytochemical screening was conducted on the extracts, and antimicrobial susceptibility testing was carried out using well diffusion method on nutrient agar and potato dextrose agar respectively. The extracts of *Tamarindus indica* were tested positive for Carbohydrates, Chloride, Taninns, Alkaloids, Flavonoids, Phlobatannins, Steroids, phenolic compounds and Saponins and the extracts of *Garcinia cambogia* had showed the presence of Carbohydrates, Vitamin C, Chloride, Alkaloids, Flavonoids, Steroids and Saponins. The aqueous, ethanol and hexanes extracts of *Tamarindus indica* had shown antimicrobial activity against *Bacillus cereus*, *Klebsiella pneumonia*, *Pencilliumnotatum* and *Aspergillusniger* and the extracts of *Garcinia cambogia* had shown antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Staplylococcusaureus* and *Pencilliumnotatum*. The ethanol extract was found to be most effective against the micro organisms. In Agarose gel electrophoresis DNA bands of 3000bp and 4000bp were obtained. Molecular weight of the *Tamarindus indica* was lower than the *Garcinia cambogia*.

Keywords: *Tamarindus indica*, *Garcinia cambogia,* Phytochemistry, Anti-microbial.

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INTRODUCTION

A majority of chemical compounds which perform vital biological functions are synthesized in plants [1]. Medicinal plants have been played an important role in human history. There are a wide variety of chemical compounds that are synthesized by plants used to perform important function such as to defend against predators such as fungi, herbivorous mammals and insects, and to do many biological functions [2]. There are 12,000 of such compounds have been isolated and identified. Chemical compounds in plants act on the human body through processes identical to those by the chemical compounds in conventional drugs; thus it have been proved that herbal medicines do not differ largely from conventional drugs in terms of how they act on human body [3]. The information has been revealed that herbal medicines are as much as effective as conventional medicines [4]. In the current study it has been discussed about *Garcinia cambogia* and *Tamarindus indica*.

Tamarindus indica

Tamarind or Tamarindus indica L.of the Fabaceae family, subfamily Caesalpinioideae, is an important food material in many countries [5]. It has been called as multipurpose tree because almost every part of tamarind finds some use from itthat may be either medicinal nutritional. In about 50 countries in the world the tamarind tree has been cultivated and is native is from tropical Africa. Asian countriessuch as Thailand, India, Sri Lanka,Bangladesh and Indonesiaare the major production areas of tamarind around the world. Costa Rica and Mexico are the biggest producers of tamarind in American continent [6]. The tamarind production is not in a commercial scale in Africa but it has been used by the local people for many purpose such as to treat diseases and for food purpose.Gambia, Senegal, Kenya, Zambia and Tanzania these are the low tamarind producing countries in Africa [7].

Garcinia cambogia

The Malabar tamarind or Garciniagummi-gutta (L.), commonly known by its previous scientific name *Garciniacambogiais* native to Southeast Asia [8]. The fruit is commonly used as a flavoring agent, food-bulking agent or food preservative, and as a traditional remedy to treat piles, constipation, rheumatism, irregular menstruation, intestinal parasites and oedema in many Asian countries [9]. Earlier phytochemical reports on the plant led to the isolation of various benzophenones, xanthones and organic acids, as major constituents and numerous scientific studies have indicated biological activity such as anticancer activity, anti-obesityand numerous othersactivities [10].

Malabar tamarind is a tropical tree which is found mostly in India, Malaysia, Africa and Sri Lanka,belongs to the family ofClusiaceae [11]. There are around 200 speciesof thegenus Garcinia, throughout the world. Out of the 200 species, 36 specieswere reported from India.Trees normally grows to a heightof 20 metres have rounded crown. The branches are soft and thin, horizontal or drooping. Leaves are shining, dark green and ovate in shape. Fruits are ovoid in shape, red or yellow in colour with 6-8 grooves that contains 5 to 8 big seeds which are surrounded by a succulent aril [12].

MATERIALS AND METHODS

Sample collection and Identification

The fruit samples of *Tamarindus indica* and *Garcinia combogia* were collected from Trissur, Kerala. The fruits were sent for proper identification. The fruits were authenticated by eminent botanist of Noorul Islam Centre for HigherEducation, Noorul Islam University, Tamilnadu, India were shown in Fig 1.

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Figure 1: Fruit samples of *Tamarindus indica* and *Garcinia combogia*

Preparation of Fruit Extracts

The fruit samples of *Tamarindus indica* and *Garcinia combogia* wereseparated and cleaned well with tap water. Cleaned fruitswere then driedunder shade. The drying was done until all the watermolecules evaporated and fruits became well-dried forgrinding. After drying, the fruits were ground well usingmechanical blender into fine powder and transferred intoair-tight container with proper labeling for further use.The dried and powdered samples of *Tamarindus indica* and *Garcinia combogia* wereextractedsequentially with Aqueous, Ethanol and Hexanes using Soxhlet apparatus. Solute thus separated werecollected in a centrifuge tube and used for furtherstudieswere shown in Fig 2.

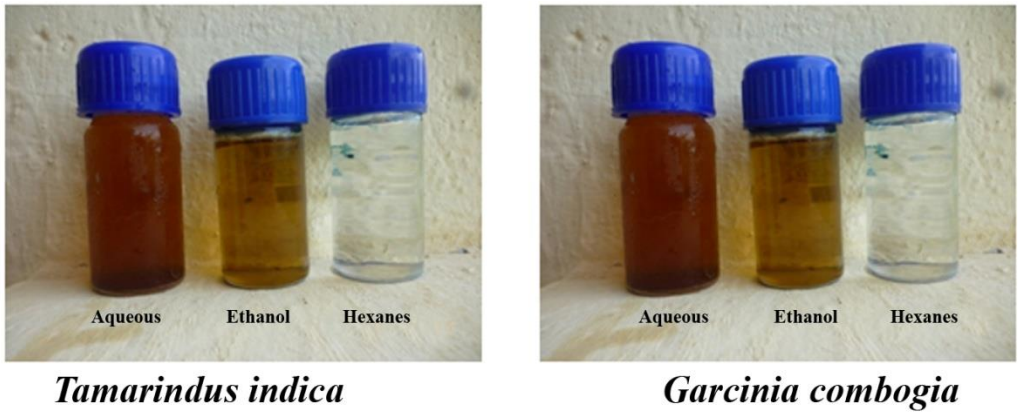

Figure 2: Fruit extracts of *Tamarindus indica* and *Garcinia combogia*

Qualitative Biochemical Screening of Secondary Metabolites

The fruit extracts of *Tamarindus indica* and *Garcinia combogia*were screened fordifferent phytochemical constituents' viz., Carbohydrates, Amino acids, Proteins, Vitamin C,Chloride,Tannins, Alkaloids, Flavonoids,Phlobatannins, Phenolic compounds, Steroids and Saponins.Phytochemical screenings of the extracts were carried outby the standard methods.

Evaluation of Antimicrobial Activity of Fruit Extracts

Culture and Media Preparation

The three different solvent extracts of the fruit samples were tested for antimicrobial activity using well diffusion assay. The microbial strains used for current study are *Escherichia coli, Klebsiella pneumonia,*

*Staphylococcus aureus, Bacillus cereus, Pencilliumnotatum, Aspergillusniger.*Themicroorganisms were collected from the Microbial TypeCulture Collection (MTCC), Chandigarh, India andmaintained in the laboratory by periodic subculture.

Antibacterial Assay

The bacterial strains obtained were inoculated in a test tube containing 5 ml of nutrient broth and the test tubes were incubated at 37ºCfor 24 hours and were referred to as seeded broth.About 20ml of the prepared Nutrient agar and Potato dextrose agar were poured into a set of well labeled sterile Petri plates under aseptic conditions and were allowed to solidify.1 ml of seeded broth were swab cultured over the solidified agar surface and six wells of 6 mm were prepared on the plate and 200µl of extracts were added to the wells. Then left the plates for 1 hour and subsequently incubated at 37ºC for 24 hours. The diameter of inhibition zones were observed, measured and photographed.

Isolation of genomic DNA

The two fresh leaves of *Tamarindus indica* and *Garcinia combogia*were collected and used for isolation of genomic DNA were shown in Fig 3. Around 2 gm of the leaf tissue was crushed using mortar and pestle and is homogenized with 2 ml of the extraction buffer.The homogenate was transferred to a centrifuge tube and equal volume of phenol: chloroform: Isoamylalcohol (25:24:1) was added to the tubes and mixed gently.For 15 minutes, the tubes were centrifuged at room temperature at 15,000 rpm. The upper aqueous phase was collected in a new tube and an equal volume of chloroform: Isoamylalcohol (24:1) was added and mixed.The upper aqueous phase obtained after centrifugation, at 15000 rpm, at room temperature for 10 minutes, was transferred to a new tube. By adding 0.1 ml of 3 M Sodium acetate (pH 7.0) and 0.7 ml of Isopropanol, the DNA was precipitated from the solution.The tubes were centrifuged at 4ºC for 15 min at 15,000 rpm, after 15 min of incubation at room temperature. The DNA pellet was washed twice with 70% ethanol and then very briefly with 100% ethanol and air dried.The DNA was dissolved in TE buffer.To removes RNA, 5µl of DNase free RNAseA (10 mg/ml) was added to the DNA.

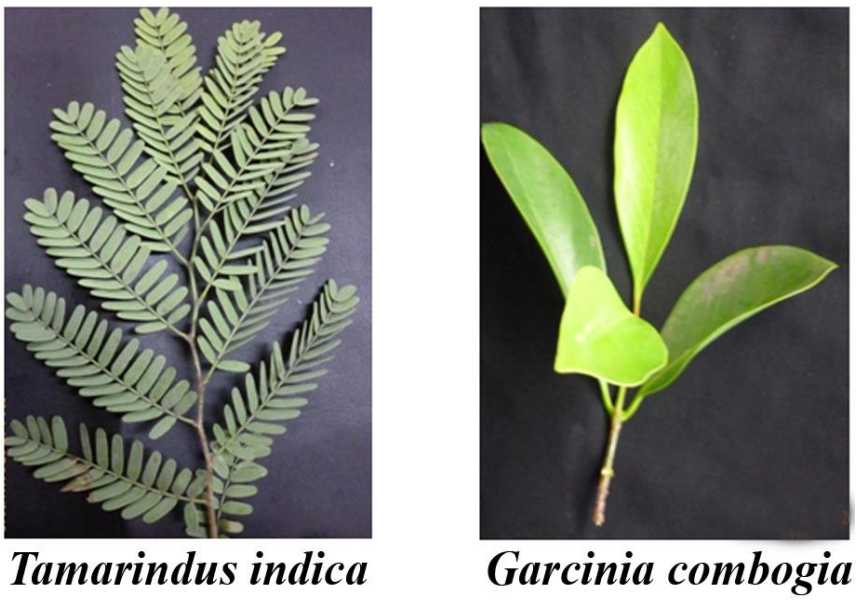

Figure 3: Fresh leaves of *Tamarindus indica* and *Garcinia combogia*

Estimation of DNA by Agarose Gel Electrophoresis

TBE buffer is the buffer system used for the separation of nucleic acids in Agarose gel electrophoresis.Sterilize the stock solution by autoclaving. 10mg of ethidium bromide was weighed in to a sterile tube and is dissolved in 1ml of distilled water. The stock solution was stored at 4ºC. 10ml of 100% glycerol or 10ml of 60% sucrose was autoclaved.1ml of 3% bromophenol blue was prepared in a sterile tube

with sterile distilled water.Sample loading dye was prepared by mixing 0.7ml of glycerol or sucrose (60%) and 0.2ml of 10X TBE and 0.1ml of 3% bromophenol blue solution.1.5µl of the sample loading dye was mixed with 10µl of DNA sample and loaded.0.24g of Agarose was weighed and sprinkled in to 30ml of 1X TBE buffer in a 100ml Erlenmeyer flask. For about 15 minutes, the Agarose was boiled to dissolve by placing the flask in a boiling water bath. The flask was removed from the water bath when the Agarose was completely dissolved and left at room temperature to cool. The platform was washed with distilled water and then wiped dry with tissue paper. The open ends of the platform are sealed securely with cello tape. The comb was placed 1 cm away from the top end and make sure that teeth of the comb do not touch surface of the platform. The platform was placed on a smooth horizontal surface. Once the Agarose solution was cooled to above 50 ˚C, the solution was poured gently to cover the entire surface of the platform and let undisturbed for about 30 minutes. Once the gel was formed, the comb was removed by gently pulling up and the cello tape is also removed from both ends. Then the gel was placed along with the platform inside the gel tank and electrophoresis buffer was poured through one side of the tank to just cover the gel surface.

The DNA sample was mixed with the loading dye and using a capillary tube, the mixture was loaded into the well carefully. Once the sample was loaded into the well, the cathode was connected towards the top end of the gel and towards the bottom end of the gel, the anode was connected. By switching on the D.C Power pack, the electrophoresis was started. The gel was run at approximately 5 v/cm. As the bromophenol blue (the tracking dye) has moved to ~1 cm from the bottom end, the current was switched off. The power supply was then disconnected and the gel along with the platform was stained in a plastic tray containing 100ml ethidium bromide(0.5µg/ml) in distilled water(Gloves are to be used while handling ethidium bromide). After 30-45 minutes, the platform and the gel were rinsed with distilled water. By keeping the platform in a slanting position, the gel was gently pushed on to UV trans- illuminator. Finally, the Ultra Visible light wasthen switched on and the bands of DNA were seen and were photographed using an orange filter.

RESULTS AND DISCUSSION

The phytochemical activities of *Tamarindus indica*and *Garcinia combogia*are studied and the results were shown in Table 1.The Aqueous, Ethanol and Hexane extracts of *Tamarindus indica* had showed the presence of Carbohydrates,Chloride,Taninns,Alkaloids,Flavonoids,Phlobatannins,Steroids, phenolic compounds and Saponins [13]. The Aqueous, Ethanol and Hexane extracts of *Garcinia cambogia* had showed the presence of Carbohydrates, Vitamin C, Chloride, Alkaloids, Flavonoids, Steroids and Saponins [14].

The Ethanol extract of *Tamarindus indica* had showed antimicrobial activity against *Bacillus cereus, Klebsiella pneumonia, Aspergillus niger* where as aqueous and ethanol extracts had showed antimicrobial activity against *Pencillium notatum* were shown in Fig 4 and Table 2.The Aqueous, Ethanol, Hexanes extracts of *Garcinia cambogia* had showed antimicrobial activity against *Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Staplylococcus aureus* whereas only hexanes extract had showed antimicrobial activity against *Pencillium notatum* were shown in Fig 5 and Table 2.DNA bands of 3000bp and 4000bp were obtained and showed in Fig 6. Molecular weight of the *Tamarindus indica* is lower than the *Garcinia cambogia* [15].

CONCLUSION

The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines mostly plant drugs [16]. The phytochemical analysis of *Garcinia cambogia* has showed positive results for Carbohydrates, Vitamin C, Chloride, Alkaloids, Flavonoids, Steroids and Saponins and the extracts of *Tamarindus indica* had showed the presence of Carbohydrates, Chloride, Taninns, Alkaloids, Flavonoids, Phlobatannins, Steroids, phenolic compounds and Saponins [17]. The Aqueous, Ethanol, Hexanes extracts of *Garcinia cambogia* had showed antimicrobial activity against *Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Staplylococcus aureus* whereas only hexane extract had showed antimicrobial activity against *Pencillium notatum*. Only the Ethanol extract of *Tamarindus indica* had showed antimicrobial activity against *Bacillus cereus, Klebsiella pneumonia, Aspergillusniger* whereas aqueous and ethanol extracts had showed antimicrobial activity against *Pencillium notatum*. The molecular weight was determined by Agarose Gel Electrophoresis (AGE) and DNA bands of 3000bp and 4000bp were obtained [18]. It was revealed that the molecular weight of *Tamarindus indica* was lower than the molecular weight of *Garcinia cambogia*. In this study, it was concluded that the phytochemical activity was higher in *Tamarindus indica* whereas the antimicrobial activity was high in *Garcinia cambogia*.

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S.No	Name of the Test	Aqueous		Ethanol		Hexane	
		Tamarindus indica	*Garcinia cambogia*	*Tamarindus indica*	*Garcinia cambogia*	*Tamarindus indica*	*Garcinia cambogia*
1	Carbohydrates	+	-	-	+	-	-
2	Amino acids	-	-	-	-	-	-
3	Vitamin C	-	-	-	-	-	-
4	Chloride	-	+	-	+	-	-
5	Tannins	+	-	+	+	+	-
6	Alkaloids	+	-	-	-	-	-
7	Flavonoids	+	-	+	+	+	-
8	Phlobatannins	+	+	+	+	+	-
9	Steroids	+	-	-	-	-	-
10	Phenolic compounds	-	+	+	-	+	+
11	Saponins	+	-	-	-	-	-

Table 1: Phytochemical activity of *Tamarindus indica* and *Garcinia cambogia*

S.No	Name of the Test	Aqueous		Ethanol		Hexane	
		Tamarindus indica	*Garcinia cambogia*	*Tamarindus indica*	*Garcinia cambogia*	*Tamarindus indica*	*Garcinia cambogia*
1	*Bacillus cereus*	No zone	8.5mm	5mm	15.5mm	No zone	12mm
2	*Escherichia coli*	No zone	9.5mm	No zone	13mm	No zone	12.5mm
3	*Klebsiella pneumonia*	No zone	9.5mm	7mm	14.5mm	No zone	12.5mm
4	*Staplylococcusaureus*	No zone	10mm	No zone	10mm	No zone	10mm
5	*Aspergillusniger*	No zone	No zone	10mm	No zone	No zone	No zone
6	Pencilliumnotatau m	21mm	No zone	22mm	No zone	No zone	5mm

Table 2: Antibacterial activity of *Tamarindus indica* and *Garcinia cambogiaagainst bacterial test organisms*

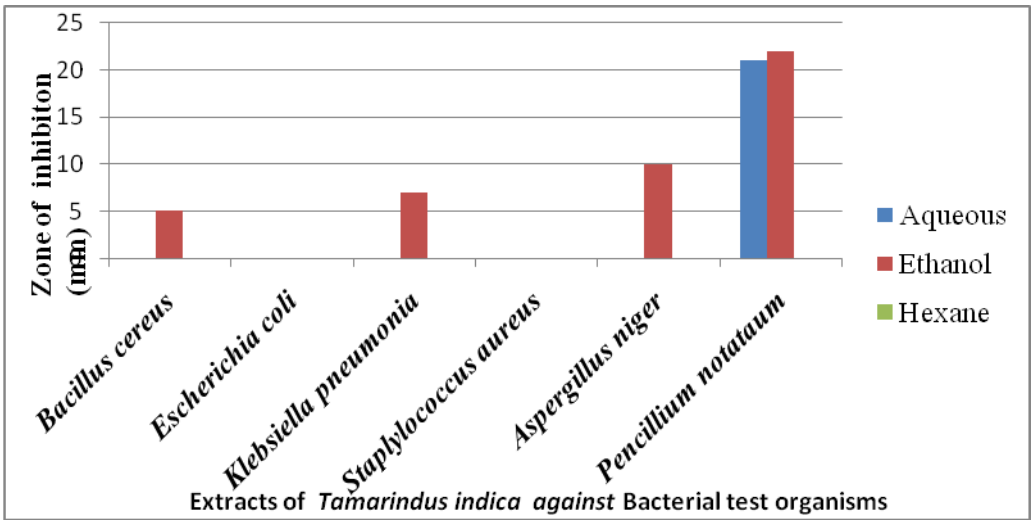

Figure 4: Antibacterial activity of Aqueous, Ethanol and Hexane extract of *Tamarindus indica* against bacterial test organisms

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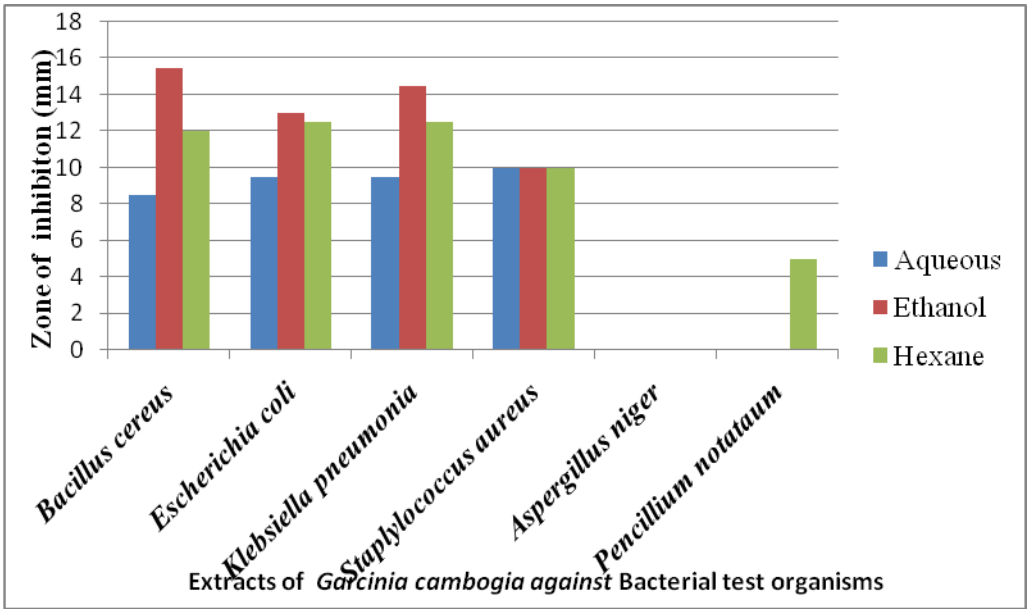

Figure 5: Antibacterial activity of Aqueous, Ethanol and Hexane extract of *Garcinia cambogia* against bacterial test organisms

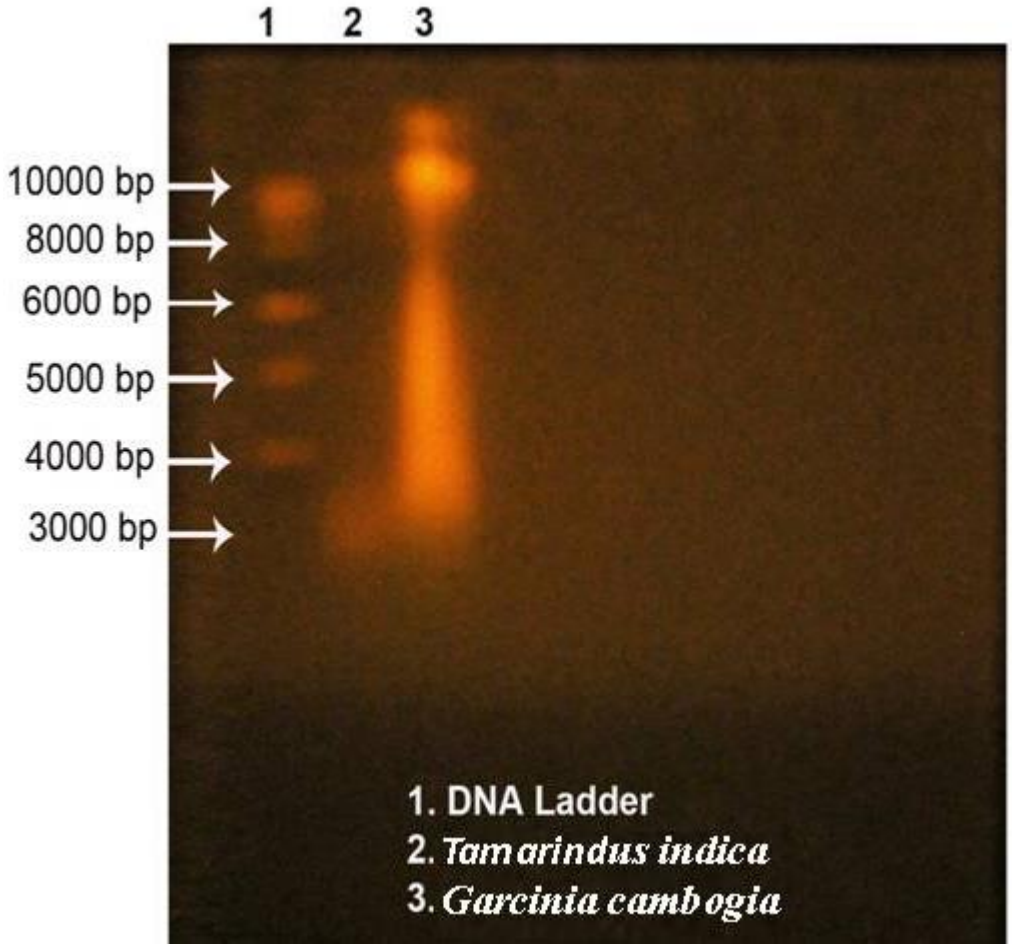

Figure 6: Agarose gel electrophoresis

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