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Full Length Research Paper

Phytochemical analysis and antimicrobial evaluation of the extracts of the Root Bark and Rachis of *Parkia biglobosa* (Jacq.) Benth. on some human Pathogens

Akintobi OA, Bamkefa BA, Adejuwon AO*, Fagbola TM

Department of Microbiology, Faculty of Sciences, Lead City University, Ibadan, Nigeria

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Various researches on the antimicrobial properties of herbal drugs are on-going due to recent reports of increase in development of resistance to synthetic drugs by pathogenic microorganisms. The aim of this work is to find the antimicrobial properties of the extract from the root bark and rachis of *Parkia biglobosa* using ethanol, petroleum ether, hexane and water. Filtered samples were tested on the following selected human pathogenic bacteria: *Pseudomonas* aeruginosa, *Salmonella typhi*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Proteus mirabilis* and *Escherichia coli*. *Agar* well diffusion technique was employed. Ethanol extracts of the root bark and rachis were observed to have antimicrobial properties on all the gram positive bacteria at minimum inhibitory concentration of 40mg/ml while hexane extract of the rachis had effect on only *Klebsiella pneumoniae* and at 40mg/ml. The phytochemical screening revealed that the root bark was richer in alkaloids, saponins, tannins, phlobatannins, flavonoids, terpenoids, steroids, cardiac glycosides and anthraquinonones. Anthraquinonones could not be extracted from the rachis of *Parkia biglobosa* by any of the solvents.

Keywords: *Parkia biglobosa*, root bark extract, rachis extract, Minimum Inhibitory Concentration, phytochemicals

Introduction

Medicinal plants are relied upon by 80% of the world's population and in India there is a rich tradition of using herbal medicine for the treatment of various infectious diseases, inflammations, injuries, and other diseases (Mann *et al*., 2008). Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs, thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines but also gives them the same potential to cause harmful side effects (Lai and Roy, 2004).

*Corresponding Author Email: ao_adejuwon@yahoo.ca

Bacterial resistance to antibacterial drugs used in the treatment of some infectious diseases has become a menace causing untold health challenges to patients. The aim of our study is to determine the antibacterial effects of the extracts of the root bark and rachis of *Parkia biglobosa* against some human pathogens in order to determine which of the selected plant parts would be the most active.

Parkia biglobosa, commonly known as the locust bean tree, African locust bean or néré, is a dicotyledonous angiosperm belonging to the family Fabaceae – Mimosoideae. It is categorized under spermatophytes which are the vascular plants (Thiombiano *et al*., 2012). It is found in a wide range of environments in Africa and is primarily grown for its pods that contain a sweet pulp and valuable seeds. Where the tree is grown, the crushing and fermenting of these seeds constitute an important economic activity. Various parts of the locust bean tree

are used for medicinal purposes (Ntui *et al*., 2012). An alcoholic extract of crude seeds has been shown to have antihypertensive activity and contractile effect on smooth muscles of the intestine.In West Africa, the bark, roots, leaves, flowers, fruits and seeds are commonly used in traditional medicine to treat a wide diversity of complaints, both internally and externally, sometimes in combination with other medicinal plants. The bark is known to be most important for medicinal uses, followed by the leaves. Medicinal applications include the treatment of parasitic infections, circulatory system disorders, such as arterial hypertension and disorders of the respiratory system, digestive system and skin. In veterinary medicine, a root decoction is used to treat coccidiosis in poultry (Hopkins and White, 1984).

Indigenous healers in Africa use different parts of the locust bean tree for health benefits. In a survey conducted on healers in Togo, *Parkia biglobosa* was one of the highest cited plants used for treating hypertension (Karou *et al*., 2011). The tree was also one of two plants listed as having real wound-healing properties in South-Western Nigeria, influencing the proliferation of dermal fibroblasts significantly. In a similar survey conducted in Guinea relating to their use of antimalarial plants, *Parkia biglobosa* was cited among those most often successfully used (Abioye *et al*., 2013). In an analysis on the antibacterial properties of the plant, another study had shown that these properties compare favourably with those of streptomycin, making it a potential source of compounds used in the management of bacterial infections (Adetutu *et al*., 2011).

Materials and Methods

Collection, Authentication and Processing of Plant Materials

The root bark and rachis of *Parkia biglobosa* used for the project work was collected from the botanical garden of the University of Ibadan, Ibadan, Oyo State. The plant materials was identified and authenticated by a Botanist at the Botany Department, University of Ibadan. The root bark and rachis were sun-dried and ground into fine powder and kept for future use.

Collection of Test Organisms

Pure cultures of the bacterial isolates used for the in vitro antimicrobial assay were obtained from the laboratory of the department of Medical Microbiology, University College Hospital, Ibadan. Oyo State. Bacterial cultures were maintained on Nutrient Agar (NA) slants for 48 hours in a refrigerator before they were subcultured into freshly prepared Petri plates for nutrient replenishment. The bacterial isolates were *Esherichia coli*, *Salmonella typhi*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Sterilization of Glasswares

The glasswares for experiments were thoroughly washed with liquid detergent and rinsed with distilled water, drained and dried before the sterilization. The glassware were wrapped with aluminum foil and sterilized at 160℃ in a hot air oven for 2hours (Fawole and Osho, 2002).

Preparation of Culture Media

Nutrient agar

According to the manufacturer's specification, the nutrient agar was prepared by weighing 28g of nutrient agar powder using a weighing balance. The agar powder was dissolved in 1000ml of distilled water inside a 1000ml conical flask. A stopper made with cotton wool wrapped with aluminum foil was used to plug the mouth of the conical flask. The solution was mixed thoroughly and then homogenized in a water bath at 80℃ for 15minutes. The conical flask containing the homogenized nutrient agar was wrapped with aluminum foil and then sterilized in an autoclave for 15minutes at 121℃ at a pressure of 15lb/sq inch (Fawole and Osho, 1998).

Validity of Test Organisms

The organisms were maintained in Nutrient Agar (NA) slants for 48 hours in a refrigerator at about 4℃ before they were further subcultured into freshly prepared Petri dishes using streak plate method. Gram staining procedure and other relevant biochemical tests such as catalase, starch hydrolysis, oxidase, urease, and indole tests were carried out on the samples to establish the validity of the test bacteria (Harrigan and McCarne, 2001).

Plant Extracts Preparation

The method of Olayemi and Opaleye (2009) was modified and employed for the analysis. Ten grams of milled extract powder of each of the plant parts (root bark and rachis) were weighed and dissolved into different 500ml beakers containing 100ml of the different solvents which are hexane, petroleum ether, ethanol and distilled water. The beaker was then covered with aluminum foil and left for 24hours during which it was stirred at 2hours interval using a glass rod stirrer. The supernatant was filtered into 100ml beaker using Whatman No.1 filter

paper. The extract was used immediately and the remaining extract stored in the refrigerator for further use.

Antimicrobial Test on Bacteria

Agar well diffusion test

The plant extracts were tested for antimicrobial activity using agar well diffusion technique. This method allowed the extract in the gel cavity to diffuse through the solid medium such that the growth of the inoculated microorganism was restricted by the efficacy of the extract constituent thereby a visible circular zone around the cavity (well) containing the extract was formed. The observed clear zone diameter was measured (Fawole and Osho, 1998).

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was done with the use of antibiotic discs (gram positive and gram negative) by the disk diffusion method (Bauer *et al*., 1966). Before each antibiotic disc was placed on each of the media surface, the pathogenic bacteria isolates from the Nutrient agar slants were streaked on each of the nutrient agar dishes after which the antibiotic disc were aseptically placed on each of the Petri plates using sterile forceps. The agar plates were then incubated at 37℃ for 24 hours. Afterward, the plates were examined for zone of inhibition. The zone of inhibition around each antibiotic disc was measured in millimeters using a transparent ruler. Antibiotic susceptibility was evaluated from the reading obtained from the diameter of the zone of inhibition. The antibiotic susceptibility patterns from each of the zones of inhibition observed on the agar plates is referred to as Antibiogram.

Inhibitory Tests

The test organism was streaked all over the surface of the solidified nutrient agar plates using inoculation wire loop. A sterile 8mm diameter cork borer was used to make a uniform deep well into the gel. Each well was then filled with 1ml of the extracts prepared in different solvents. The petri plates were allowed to stand for 30minutes at room temperature to allow proper diffusion. The control experiments were then set up using each of the solvents (without extracts). Sterilized distilled water was equally used as aqueous control sample. The plates were incubated at 37℃ for 24hours after which the zones of clearance were measured with a metre rule (Harrigan and McCane, 2001).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the agar streak technique. The concentration around the agar well that gave the least inhibition was regarded as the minimum inhibitory concentration (Bauer *et al*., 1966).

Measurement of Analysis

Different concentrations of *Parkia biglobosa* root bark extracts and rachis extracts was prepared by dissolving 10grams of powdered sample in 100ml of sterile distilled water and in each of these solvents (Ethanol, Petroleum ether and Hexane). Each was filtered, concentrated in water bath at 70℃ for 4hours and the extracts tested for antimicrobial activities against the following bacteria: *Escherichia* coli, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Standard antibiotics disc was also used on the microbial isolates and their antimicrobial activities evaluated.

Phytochemical Screening of the Root Bark and Rachis Extracts

Phytochemical screening was carried out to indicate the presence or absence of phytochemicals in the root bark extract as well as the rachis extract. The constituent phytochemicals were assayed on the powdered specimen using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989), Harborne (1973), and Edeoga *et al*., (2005). Qualitative assay of the phytochemicals screened were saponins, tannins, phlobatannins, steroids, terpenoids, anthraquinones, flavonoids, alkaloids, cardiac glycosides, reducing sugars and resins.

Results

For the root bark extract, ethanol extracts was observed to be reactive on all the gram negative bacteria but unreactive to gram positive bacteria. Hexane did not show any effect on the bacteria isolates used. Table 1 shows the minimum Inhibitory concentration of the root bark extracts (measured in millimeters) while Table 2 shows the minimum inhibitory concentration of rachis extracts. Minimum Inhibitory concentration (MIC) is defined as the minimum dilution of extract that will completely inhibit test organism. It was observed that when the root bark extracts of *Parkia biglobosa* was extracted with Hexane, it was not effective on all the bacterial isolates. Ethanol extract reacted effectively on

Table 1: The zones of inhibition (mm) of the root bark extract of *Parkia biglobosa*

Isolate used	Solvent used for extraction	Undiluted extract (100mg/ml)	Control
Escherichia coli	Ethanol	22	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Klebsiella Pneumoniae	Ethanol	21	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Pseudomonas aeruginosa	Ethanol	24	-
	Petroleum ether	25	-
	Hexane	-	-
	Aqueous	-	-
Staphylococcus aureus	Ethanol	-	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Proteus Mirabilis	Ethanol	25	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Salmonella Typhi	Ethanol	24	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Streptococcus pyogenes	Ethanol	-	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-

Key: - negative, n = 3

Table 2: The zones of inhibition (mm) of isolates with rachis extract *Parkia biglobosa*

Isolate used	Solvent used for extraction	Undiluted extract (100mg/Ml)	Control
Escherichia Coli	Ethanol	24	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Klebsiella Pneumonia	Ethanol	20	
	Petroleum ether	-	-
	Hexane	18	-
	Aqueous	-	-

Table 2. cont

Pseudomonas aeruginosa	Ethanol	34	-
	Petroleum ether	20	-
	Hexane	-	-
	Aqueous	-	-
Staphylococcus Aureus	Ethanol	-	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Proteus Mirabilis	Ethanol	22	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-
Salmonella Typhi	Ethanol	26	-
	Petroleum ether	25	-
	Hexane	-	-
	Aqueous	-	-
Streptococcus pyogenes	Ethanol	-	-
	Petroleum ether	-	-
	Hexane	-	-
	Aqueous	-	-

Key: - negative, n = 3

Table 3: The Minimum Inhibitory Concentration (MIC) of the root bark extract of *Parkia biglobosa*

Isolate used	Solvent used for extraction	Undiluted extract (100mg/ml)	10ml + 5ml (66.7mg/ml)	10ml+10ml (50mg/ml)	10ml+15ml (40mg/ml)	Control
Escherichia coli	Ethanol	22	20	18	15	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Klebsiella pneumoniae	Ethanol	21	15	13	12	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Pseudomonas aeruginosa	Ethanol	24	20	15	12	-
	Petroleum ether	25	22	18	15	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Staphylococcus aureus	Ethanol	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-

Table 3. cont

Proteus mirabilis	Ethanol	25	22	20	19	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Salmonella typhi	Ethanol	24	18	16	14	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Streptococcus pyogenes	Ethanol	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-

Key: - negative, n = 3

Table 4: The Minimum Inhibitory Concentration of rachis of *Parkia biglobosa*

Isolate Used	Solvent used for Extraction	Undiluted Extract (100mg/ml)	10ml + 5ml (66.7mg/ml)	10ml+10ml (50mg/ml)	10ml+15ml (40mg/ml)	Control
Escherichia coli	Ethanol	24	20	18	12	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Klebsiella pneumoniae	Ethanol	20	16	12	11	-
	Petroleum ether	-	-	-	-	-
	Hexane	18	16	13	11	-
	Aqueous	-	-	-	-	-
Pseudomonas aeruginosa	Ethanol	34	25	20	12	-
	Petroleum ether	20	18	15	13	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Staphylococcus aureus	Ethanol	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Proteus mirabilis	Ethanol	22	20	18	12	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-

Table 4. cont

Salmonella typhi	Ethanol	26	20	17	12	-
	Petroleum ether	25	23	20	17	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-
Streptococcus pyogenes	Ethanol	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
	Hexane	-	-	-	-	-
	Aqueous	-	-	-	-	-

Key: - negative, n = 3

Table 5: The zones of inhibition of gram negative bacterial isolates using standard antibiotics discs

Antibiotics	Code	Concentration	*Salmonella typhi*	*Escherichia* coli	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
Augmentin	AUG	30µg	-	-	-	-	-
Tetracycline	TET	30µg	-	-	-	6mm	-
Amoxicillin	AMX	25µg	-	-	-	-	-
Nitrofurantoin	NIT	30µg	13mm	12mm	10mm	-	-
Nalidixic acid	NAL	30µg	13mm	15mm	15mm	-	-
Ofloxacin	OFL	30µg	25mm	30mm	15mm	13mm	10mm
Gentamicin	GEN	10µg	13mm	13mm	-	8mm	6mm
Cotrimaxazole	COT	25µg	-	-	-	-	-

Key: **-** negative, n = 3

all gram positive bacteria isolates used. Petroleum ether was effective against only Pseudomonas aeruginosa and that is at 40mg/ml. Ethanol extract was also found to be most active against all the tested gram negative bacteria strains followed by petroleum ether extract (Tables 3 and 4).

Tables 5 and 6 show the sensitivity of the isolates to Standard Antibiotic Disc that is commercially available. The diameter of clearance was also taken to compare with the extract used. Gram positive bacteria (*S. aureus* and *S. pyogenes*) resisted Tetracycline, Chloramphenicol, Cotrimoxazole, Erythromycin and Augmentin. However, S. *aureus* was sensitive to Streptomycin and Gentamicin with 15mm and 11mm clear zones of inhibition respectively while *S. pyogenes* was sensitive to Streptomycin and Gentamicin with 19mm and 12mm clear zone of inhibition respectively. Also, S. typhi resisted Augmentin, Amoxycillin and Cotrimoxazole but sensitive to Tetracycline, Nitrofurantoin, Nalidixic acid, Ofloxacin and Gentamicin with 13mm, 13mm, 13mm, 25mm and 13mm inhibition zone respectively. *Escherichia coli* was resistant to Augmentin (AUG), Tetracycline (TET), Amoxicillin (AMX), Cotrimoxazole (COT) but sensitive to Nitrofurantoin (NIT), Nalidixic acid (NAL), Ofloxacin (OFL) and Gentamicin ((GEN) with 12mm, 15mm, 30mm and 13mm zone of inhibition respectively. *Klebsiella pneumoniae* was sensitive to NIT, NAL and OFL with 10mm, 15mm, 15mm zone of inhibition respectively but resisted to AUG, TET, AMX, COT and GEN. *Proteus mirabilis* was resistant to AUG, AMX, COT, NIT and NAL but susceptibile to TET, OFL and GEN with 6mm, 13mm and 8mm respectively. Also, P. aeruginosa resisted AUG, TET, AMX, COT, NIT and NAL but sensitive with 10mm and 6mm clear zone respectively (Tables 5 and 6).

The root back of *P. biglobosa* was assayed using water, ethanol, petroleum ether and hexane for extraction. The result in Table 7 showed that alkaloids, saponins, tannins, phlobatannins, terpenoids and cardiac glycosides were extractile with water, ethanol and petroleum ether. Hexane was able to extract only flavonoids, steroids and anthraquinonones from the root back. Similarly, the rachis extract of the *P. biglobosa* was qualitatively assayed using water, ethanol, Petroleum ether and hexane as extractants. The result in Table 8 showed that water was able to alkanoids, flavonoids and steroids from the rachis while ethanol extracted tannins, terpenoids and cardiac glycosides. On the other hand,

Table 6: The zones of inhibition of gram positive bacterial isolates using standard antibiotics discs

Antibiotics	Code	Concentration	*Staphylococcus aureus*	*Streptococcus pyogenes*
Streptomycin	STR	10µg	15mm	19mm
Tetracycline	TET	30µg	-	-
Chloramphenicol	CHL	10µg	-	-
Cotrimoxazole	COT	25µg	-	-
Cloxacillin	CXC	5µg	-	-
Erythromycin	ERY	5µg	-	-
Gentamicin	GEN	10µg	11mm	12mm
Augmentin	AUG	30µg	-	-

Key: **-** negative, n = 3

Table 7: Qualitative phytochemical screening of extracts of *Parkia biglobosa* root bark

Bioactive Constituents	Aqueous Extract	Ethanol Extract	Petroleum Ether Extract	Hexane Extract
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phlobatannins	+	+	+	+
Flavonoids	+	+	+	-
Terpenoids	+	+	+	+
Steroids	+	+	+	-
Cardiac Glycosides	+	+	+	+
Anthraquinononones	+	+	+	-

Key: + = present; - = absent

Table 8: Qualitative phytochemical screening of extracts of rachis of *Parkia biglobosa*

Bioactive Constituents	Aqueous Extract	Ethanol Extract	Petroleum Ether Extract	Hexane Extract
Alkaloids	+	-	-	+
Saponins	-	-	-	-
Tannins	-	+	-	+
Phlobatannins	-	-	+	-
Flavonoids	+	-	-	-
Terpenoids	-	+	+	+
Steroids	+	-	-	-
Cardiac Glycosides	-	+	-	+
Anthraquinononones	-	-	-	-

Key: + = present; - = absent; Key: + = present; - = absent

petroleum ether extracted phlobatannins, terpenoids while hexane extracted alkaloids, tannins, terpenoids and cardiac glycosides.

Discussions

The antimicrobial activity of the solvent extracts of the root bark and rachis of *Parkia biglobosa* against the human pathogenic bacteria has revealed the degree of potency of root bark extracts as observed in the values of inhibition zones shown in tables 1 and 2. The result obtained showed that for the root bark petroleum ether extract has significant antimicrobial activity against Pseudomonas aeruginosa only, while ethanol extract has antimicrobial activity on all the gram negative pathogenic bacteria under test; these strains are Pseudomonas aeruginosa, Salmonella typhi, *Klebsiella* pneumoniae, *Proteus mirabilis* and *Escherichia* coli. For the rachis, petroleum ether extract showed significant antimicrobial activity against two of the pathogens which are Pseudomonas aeruginosa and Salmonella typhi while ethanol extract has antimicrobial on all the gram negative bacteria tested and hexane extract showed antimicrobial activity against *Klebsiella* pneumoniae alone.

This result showed that both the root bark and rachis extracts were effective only against the gram negative bacteria. Ethanol extract was also found to be most active against all the tested gram negative bacteria strains followed by petroleum ether extract (Tables 3 and 4). This is similar to the findings of Harborne (1973) who reported that alcohol was the best organic solvent for the extraction of most plant bioactive principles of medicinal importance. This also tallies with the findings of Millogo-kone *et al* (2008) who showed that the ethanoic extract of the root bark of *Parkia biglobosa* was very active against gram negative bacteria. Furthermore, Millogo-kone *et al*., (2008) later showed that alcoholic extract of the root bark and rachis was the most active against all his tested gram positive bacteria. The phytochemical screening revealed that the root bark was richer in alkaloids, saponins, tannins, phlobatannins, flavonoids, terpenoids, steroids, cardiac glycosides, and anthraquinononones. This is in accordance with the research findings of Edeoga *et al*. (2005) and Sofowora (2008) who worked on the phytochemical constituents P. *biglobosa* and P. bicolor to indicate the presence or absence and the amounts of phytochemicals in the root bark extract respectively.

Conclusion

The extracts of root bark and rachis of *Parkia biglobosa* were very active against all the gram positive bacteria. The results showed that the root bark and rachis, when extracted with ethanol are much more active and potent as the decoction of stem prescribed by the traditional healers. If this can be developed and purified it would assist the government's aim at producing herbal drugs that will be potent and affordable by everyone.

Recommendation

The root bark and rachis of *Parkia biglobosa* should be investigated further for the formulation of antibiotics for cure of pathogenic bacterial infections. In doing this, ethanol may be preferred than any other solvents for the extraction of bioactive material or chemical from any part of medicinal plants.

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