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A preliminary screening on pharmacognostic, phytochemical and *In-vitro* antibacterial activity of *Mangifera indica* seed kernel L.,

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Abstract
Mangifera indica is a common ayurvedic plant whose parts are often used for a variety of reasons. In India, seed kernels are used to treat diarrhoea, dysentery, ulcers, and other disorders. The purpose of this research is to find out more about the ethno medicinal and bioactive components properties of M.indica seed kernels, as well as to establish phytochemical and pharmacological standards for them. According to reports, the quality of the raw medicine under research serves as a pharmacognostical benchmark for the drug seed kernel. Results showed the MISK powder yielded 7.2% total ash, acid insoluble ash of 2.8 % and water soluble ash of 3.7%. The major secondary metabolites present in both the aqueous and phenolic extracts of MISK were alkaloids, tannins, flavonoids, and phenolic compounds. The total microbial assay revealed that microbial load and other microorganisms were present within ayurvedic pharmacopoeial limits. 200 µg of MISKPE shows good antibacterial activity against Eschericia coli, Salomonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pyogens. The pharmacological and phytoconstituent parameters revealed in this study could be used as primary criteria for a crude drug's identity, quality, and purity.

Keywords: pharmacognosy, *M. indica* seed kernel, phytochemical, total ash

Introduction
Herbal medicine has experienced exponential growth in recent years, and these treatments are gaining appeal in both developing and underdeveloped nations due to their natural origins and lack of negative effects. Today the Majority of traditional remedies are made out of medicinal plants and organic substances. The World Health Organization (WHO) has compiled a list of 21,000 medicinal plants used around the world [1]. Approximately 30% or more of today's pharmacological medications are produced either directly or indirectly from plants [2].
The investigation of medicine derived from natural sources is called pharmacognosy. It is involved in the examination of crude pharmaceuticals, as well as consistency and authentication. Pharmacognosy has also been used to identify well-known plant species and to verify the authenticity of commonly used traditional medicinal herbs using phenotypic, phytoconstituents, and physicochemical analysis. The significance of pharmacognosy has been widely recognized. Despite taxonomic identification, pharmacognostic review includes aspects that assist in the detection of adulteration in dry powder form. This is essential because once the plant parts are powdered; they lose their morphological identity and are susceptible to adulteration [3]. *Mangifera indica* L. is one of the most popular fruits that belong to the family Anacardiaceae. The mango fruit is reckoned as the best among all indigenous fruits. All parts of *M. indica* are frequently used in the Indian system of medicine for a broad diversity of remedies. Leaves, flowers, bark, unripe and ripe fruit are acrid, coolent and astringent to the bowels and have been employed to cure "vata", "pitta" and "kapha". *M. indica* has also been traditionally used for the treatment of leucorrhoea, dysentery, bronchitis, biliousness, urinary discharges, throat troubles, vaginal troubles, cough, ophthalmic eruptions, asthma. It is also used as an aphrodisiac, tonic, appetizer, beautifier of the complexion, laxative, diuretic, stomachic, and antisyphilitic [4]. The seed kernel of the *M. indica* contributes up to 18-22% of the fruit and has not been used so far, remaining as waste. It is also an abundant source of phenolic compounds. Conventionally, MISK is used to cure diarrhoea, wound healing, to expel tapeworms and ulcers [5]. MISK showed anti-diarrhoeal activity, antioxidant activity, and chelating activity [6]. It is observed that people of India uptake MISK in roasted form during starvation. Its powder is invoked as an astringent in bleeding piles. Communicable diseases are the major cause of morbidity and mortality among people [7]. There are four different types of diseases that are caused in humans, such as pathogenic diseases, insufficiency diseases, genetic diseases, and physiological diseases. Pathogenic diseases can also be classified as communicable or non-communicable. All these diseases are caused by the action of microbes or their toxins and are considered a major threat to human society. In recent days, pathogenic diseases like gastroenteritis (diarrhoea, dysentery), peptic ulcers, and metabolic disorders due to stress, cancer, etc., are frequently encountering humans. Different bacterial agents like *Escherichia coli, Klebsiella pneumoniae, Staphylococcus sp, Salmonella sp,* and *Pseudomonas sp* cause

respiratory and gastrointestinal diseases. Some of the causative agents have the ability to exist in cruel conditions owing to their multiple ecological habitats [8]. Most of these organisms have multidrug resistance characteristics, which will be hard to cure. As a result, the pharmacognostic and physiochemical properties of MISK crude powder were investigated in this study to better understand its purity and antibacterial activity.

Materials and Methods

Based on its traditional employment, the *M. indica* seed kernel was chosen to investigate its biopotential. The *M.indica* seed kernel was obtained in Tiruchirappalli, Tamil Nadu's Thathachariar garden. Only the Neelum variety was chosen and evaluated for biopotential characteristics. The plant material was authenticated by Dr. John Britto, Professor, Department of Botany and Director, Rapinat Herbarium, St. Joseph's College, Thiruchirapalli, Tamilnadu.

Processing of *M.indica*

A sterilised knife was used to peel the fresh *M. indica* fruit. The soft embryonic section of the seed was detached and the shade dried completely. Using an electric grinder, the dried seed kernel was pulverised. For future research, the seed kernel powder was kept in a sterile container.

Preparation of Aqueous extract of MISK

The MISK powder was combined with sterile water and stored for 72 hours. It was filtered through a muslin cloth before being compacted in a hot air oven at 50˚C. For future usage, the aqueous extracts were stored in a sterile container and refrigerated [9].

Preparation of Phenolic extract of MISK

Soxhlet extraction was used to obtain the phenolic extract of MISK. 50gm of plant material, ethanol and methanol in a 1:1 ratio. Extraction took place at 90°C for 12 hours. The extracts were then filtered via Whatmann filter paper under a vacuum (No. 1). To remove the solvent, the extract was dried in a vacuum evaporator [10].

Processing of *M.indica* seed

The fresh seed was cut to separate the kernel from the shell and fixed in FAA (5ml of Formalin + 5ml of Acetic acid + 190 ml of 70% Ethyl alcohol). After 24 hours of fixing, the infiltration of the sample was accepted by gradual addition. The specimens were cast into paraffin blocks [11].

Sectioning of MISK

A rotary microtome was used to segment the paraffin-embedded material. The wax used to have a thickness of 10-12 mm. The portions were dewaxed according to the standard method [12]. The sections were stained with toluidine blue [13]

Photomicrographs of MISK

Micrographs are used to supplement microscopic descriptions of seed kernels as appropriate. Nikon Microscope was used to take photographs at various magnifications. Polarized light was used to analyse crystals, starch grains, and lignified cells. Descriptive terms of the anatomical features are noted as given in the standard anatomy books [14].

Pharmacognostic studies of MISK

Organoleptic Character Evaluation [15]

Macroscopic investigations of the *M.indica* seed kernel included organoleptic characteristics of the drugs such as colour, odour, taste, texture, etc.

Physicochemical parameter analysis

According to the Indian Pharmacopoeia, numerous physicochemical characteristics such as foreign matter, total ash, acid insoluble ash, water soluble ash, water soluble extractive value, and alcohol soluble extractive value were calculated [16].

Fluorescence analysis

After treatment with various solvents such as chloroform, hexane, benzene, ethanol, ethyl acetate, acetone, 50 % sulphuric acid, 10% sodium hydroxide (aqueous and alcoholic), and dilute hydrochloric acid, powdered seed kernels were analysed under visible and ultra violet light for colour change [17]

Determination of microbial index

Powdered seed kernel was subjected to analysis the Total viable aerobic count, Total viable fungal count and test for enteric pathogens.

Antibacterial study of *M.indica* seed kernal extracts

A known quantity of aqueous and phenolic extracts were dissolved in a 1:1 DMSO:Methanol mixture. This was then diluted with phosphate buffered saline in an equal volume (PBS pH 7). It was then sterilised with a sortorious syringe filter with a pore size of 0.22µm. The extract was loaded into sterile discs with a concentration of 50µg to 250µg per disc and dried. Solvent fixed discs were also prepared as a negative control. On Hi-Media discs, oxytetracycline served as a positive control.The antibacterial activity of MISK extract was determined using the disc diffusion method [18]. Four-hour-old fresh cultures of clinical isolates are seeded on Mueller-Hinton agar plates. The MISKAE and MISKPE Loaded discs were dispersed on agar plates containing test organisms. The plates were incubated for 24 hours at 37°C to observe the zone of inhibition.

Assay on MIC value of MISK extracts

The Minimal Inhibitory Concentration was determined using the agar dilution method [19]. The stock concentration of MISK extracts was made using a 1:1 ratio of DMSO: Methanol, which was then diluted with an equivalent amount of phosphate buffered saline (pH 7). A sterile mueller hinton agar was prepared and kept molten until needed. In separate tubes, 20ml of medium be combined with known concentrations of MISK aqueous and phenolic extracts. This mixture was carefully swirled to ensure that the extract and medium were completely mixed before being poured onto the plate. After it had solidified, it was inoculated with both the test and standard microorganisms. The plates were incubated at 37°C for 24-48 hours. MIC was recorded based on the growth of the organisms.

Qualitative and quantitative phytochemical screening of MISK extracts

Standard methods were also used to detect the existence of phytochemical constituents in freshly prepared extracts [20].

Result and discussion

In the past few years different diseases caused by microbes has remained a major cause of death and the number of individuals living with diseases was continuing to expand. Due to the noxious and adverse side effects of synthetic drugs, conventional management has failed to provide the fullest cure for illness. Medicinal plants have been used to treat diseases all across the world for thousands of years. Herbal medicine is making a comeback in order to better meet our current and future health demands. Herbal medicine is founded on the conviction that plants contain natural substances that can assist individuals in staying healthy and improving their health. According to the WHO, traditional medicine is used by up to 80% of the world's population, while ayurveda and medicinal plants are used by 65% of the Indian population in rural regions to satisfy their basic health needs. India has a gold mine of well-documented and well-practiced traditional herbal medical expertise. *Mangifera indica* seed kernel is most commonly used by tribal peoples of India. Keeping in view of the ethano pharmacological importance of *M. indica* seed kernel, this study was undertaken for better utilization of MISK. Seed kernel has a brownish white appearance, a mild aromatic odour, and a bitter flavour. The seed kernel has the appearance of a 70-hour chick embryo. M. indica seed kernel length was 5.5±0.89 µm and width was 2.13±0.7 µm. The seed kernel had a coarse texture and a very smooth fracture.

Anatomy of *Mangifera indica* seed kernel

The seed consists of a thin seed coat and two thick cotyledons. The seed coat in slightly thick end along the raphe and becomes uniformly thin in other parts of the seed coat (Fig 1). The raphe is 1.5 mm thick and it consists of loosely arranged thin walled parenchyma cells. Along the inner part of the raphe occur many irregular masses of vascular strands and it located in tangential band. The strands have thick walled lignified squarish cells. The strands are variable in shape and size (Fig 1 & 2).

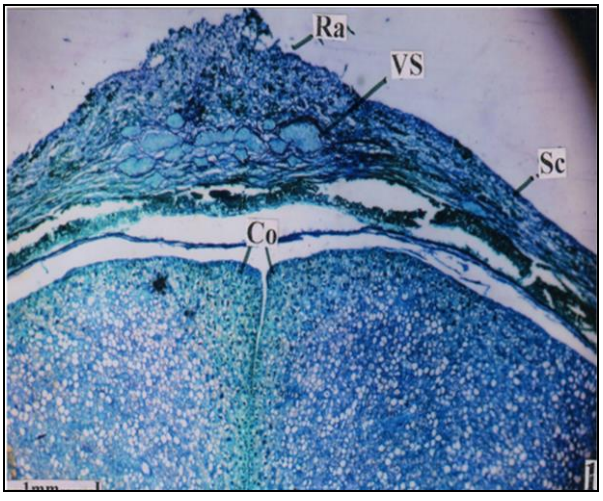

Fig 1: Ts of seed through raphe of the seed coat and cotyledons.

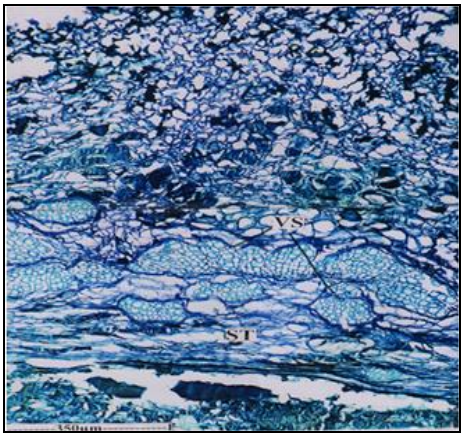

Fig 2: Ts of raphe enlarged showing the vascular strand (Co – cotyledon, ra- raphe, SC- Seed coat, ST- sarco test VS- vascular Strand).

The cotyledons are thick and fleshly. They have homogenous, thin walled compact parenchyma cells which are angular or circular in shape. The epidermal layers of the cotyledons are distinct. The outer epidermis of the cotyledons includes thick walled, small, squarish cells. The inner epidermis of the cotyledons includes narrow, cylindrical slightly thick walled cells (Fig 3, 4 & 5). Wide, large, circular laticiferous canals are frequently seen in the cotyledons. Surrounding the canals occur wide zone of the xylem and phloem elements (Fig. 6). The canals are long narrow and unbranched. They include dark amorphous tannin content and cylindrical starch grains (Fig. 7). The ground parenchyma cells have dense deposition of tannin bodies and starch grains (Fig. 8 & Fig. 9). Circular darkly stained tannin bodies are also seen in the powder. The tannin bodies `are variable in size (Fig. 10.). The xylems elements have annular and spiral lateral wall thickening (Fig.11.). There are thick bundles of xylem elements and lactiferous canals associated with vascular strands (Fig. 11). The xylem is annular, spiral and reticulate elements. Large starch grains of various size and shape are rich in the powder. The grains are mostly simple type. Some of the grains are circular in outline with "+"shaped Polari mark. There are also elliptical or ovoid starch grains which possess 'y' shaped Polari mark. The circular starch grins are 20 µm in diameter. Elliptical starch grains are 50 µm long and 25 µm thick (Fig. 12, 13 & 14).

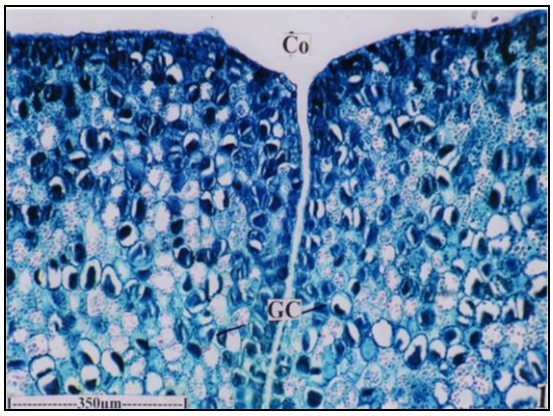

Fig 3: T.S. of Cotyledon – a Portion enlarged

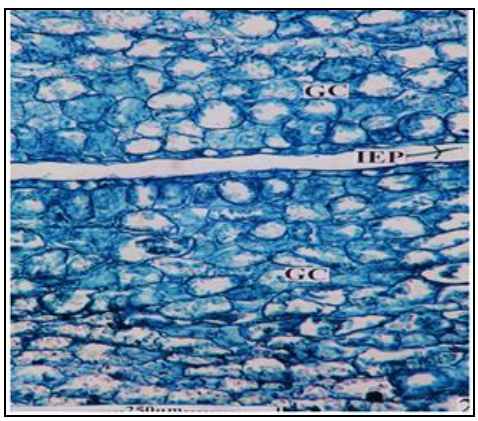

Fig 4: T.S of cotyledon showing inner epidermal layers (CO- cotyledon, GC- ground cells, IEP- Inner epidermis OEP- outer Epidermis)

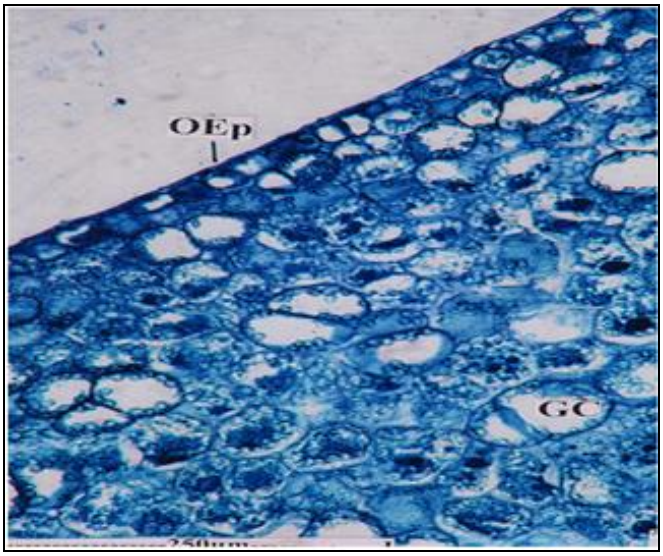

Fig 5: T.S of cotyledon showing outer epidermal (CO- cotyledon, GC- ground cells, IEP- Inner epidermis OEP- outer Epidermis)

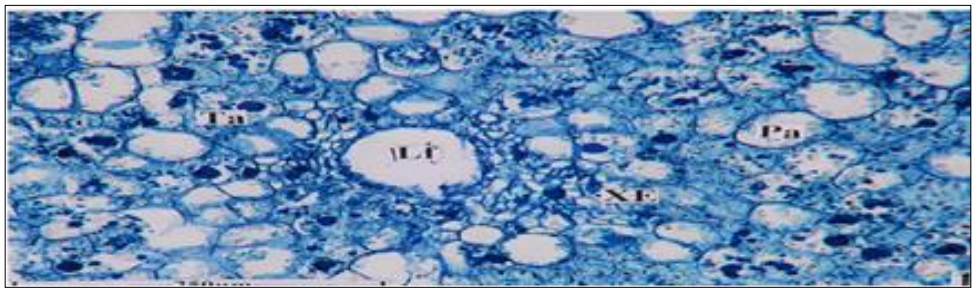

Fig 6: T.S of cotyledon showing laticiferous canal ensheathered by vascular tissue

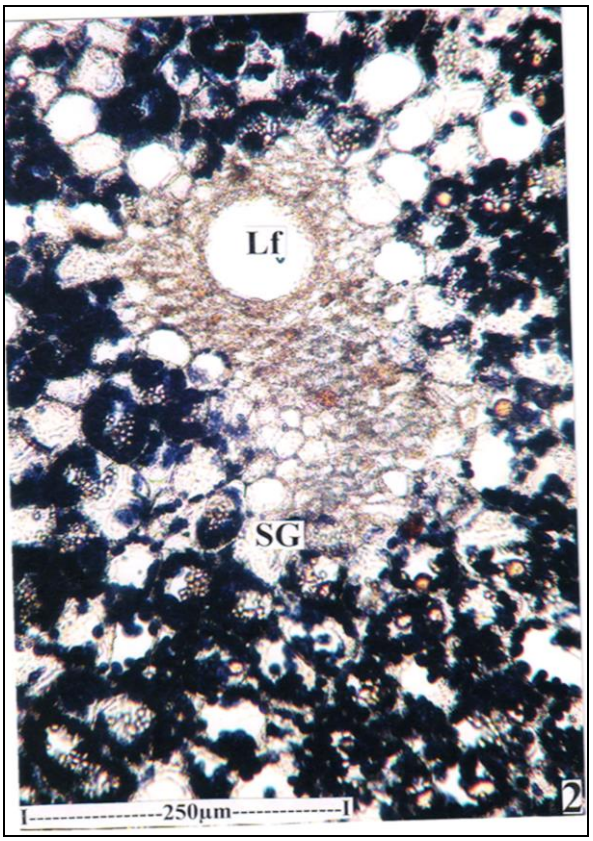

Fig 7: Cells of the cotyledon stained with IKI sowing dense accumulation of starch

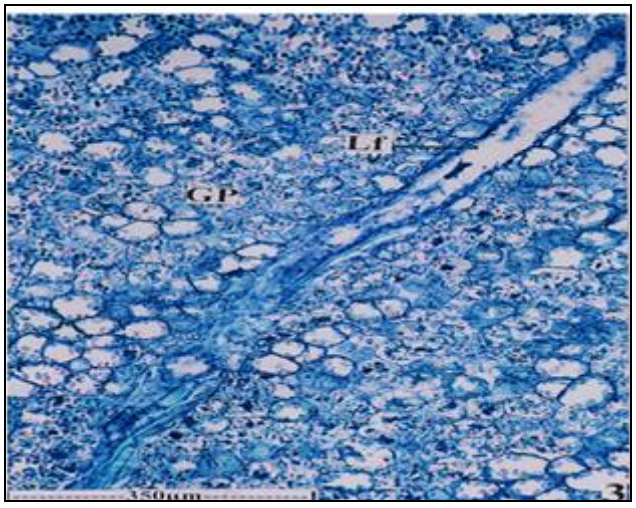

Fig 8: Secretory canal in laongitudinal sectional View (GP- Ground parenchyma, LF- latici- fer PA- parenchyma, SG- starch grains, TA- tannin, XE- xylem element

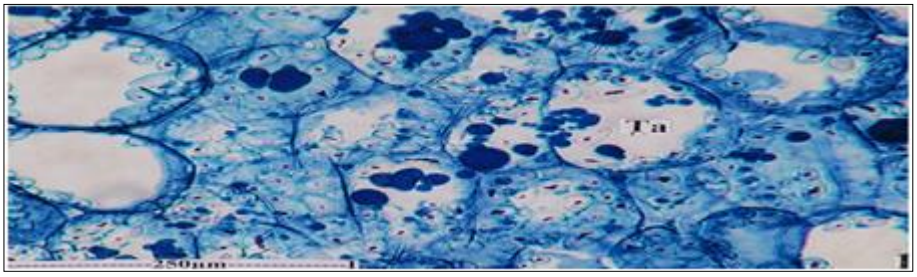

Fig 9: Spherical tannin in the cells of the cotyledon

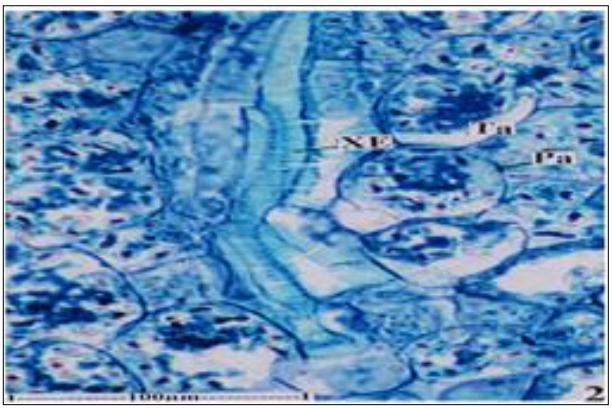

Fig 10: Xylem strands showing lateral wall thickenings

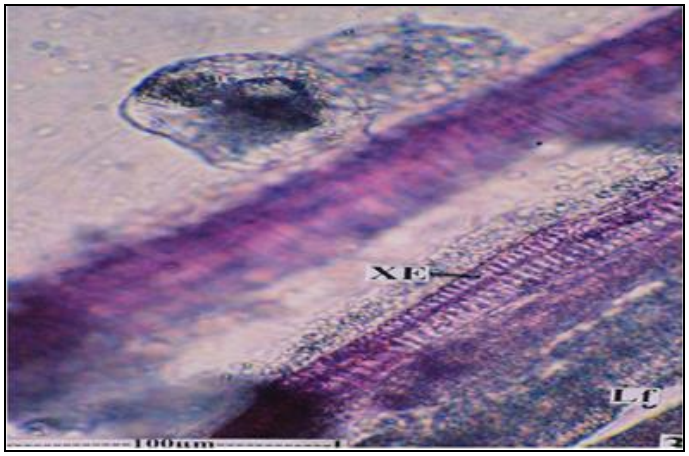

Fig 11: Powder of the cotyledon showing vascular strands (pa-parenchyma, Ta- tannin, XE- xylem element, Lf- laticifer

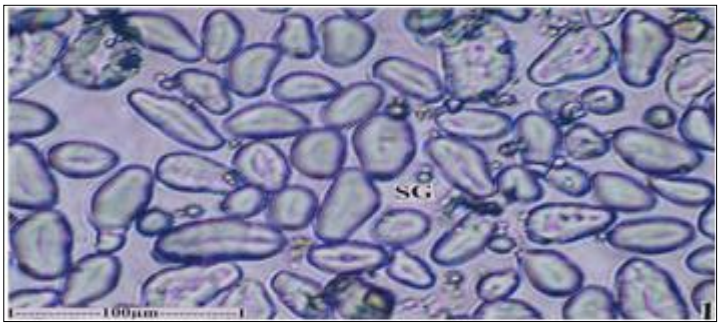

Fig 12: Seed powder showing starch grains of deifferent shape of size.

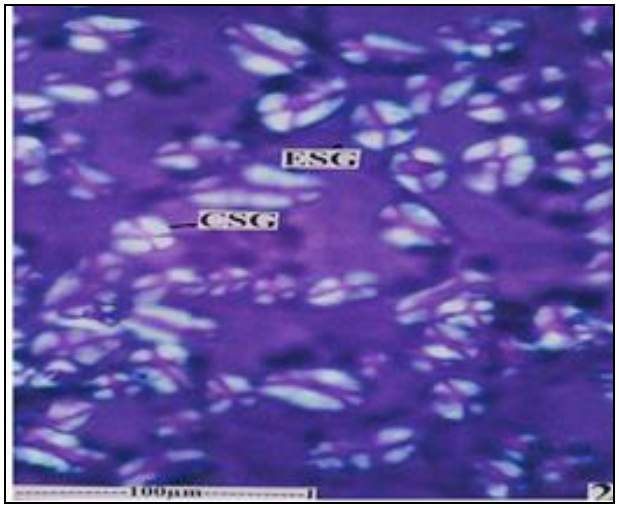

Fig 13: Starch gains as viewed under polarized light.

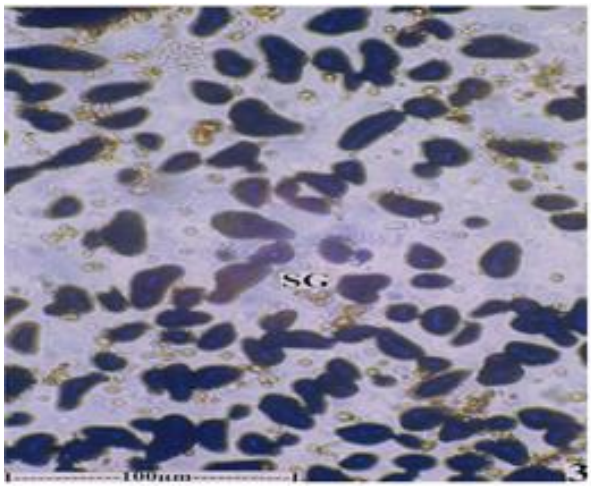

Fig 14: Starch grains stained with IKI (CSG – concentric starch grain, ESG- Excentric starch grain, SG- starch grain

Table 1. Showed the physiochemical analysis of MISK powder it is free of foreign substances. The amoun of foreign matter is directly proportional to the ash content of the powder material. Total ash, acid and water insoluble ash, were all within the confines of the Indian ayurvedic pharmacopoeia. The total ash content of MISK powder was 7.2 %, 3.7 % water soluble ash and 2.8% acid soluble ash. The percentage of water soluble extractive in powder was higher (25.7%), followed by the percentage of alcohol soluble extractive (22 %). The higher extractive levels showed the plant had more polar chemicals, such as phenolic compounds. The current investigation on the pharmacognostical characteristics of *M. indica* seed kernels provides important information for finding the proper identity and distinguishing real drug sources from closely similar species. The total ash value is primarily essential for metal, salt, and silica detection (Musa *et al*., 2006) [21]. The drug employed in this investigation had a very low ash value, indicating that it was free of extraneous materials. Acid Insoluble ash is a component of total ashes that is primarily composed of silica and indicates contamination by earthy material. The quality and purity of crude drugs were determined using ash values. Indirectly, the presence of an inorganic ingredient in the raw drug suggests the presence of water soluble ash. The extractive values were helpful in

determining the chemical components of the crude drug [22]. The presence of highly polar compounds such as flavonoids, protein, carbohydrates, and others was indicated by the increased water-soluble extractive value. The presence of greater polar substances such as phenolic compounds and other alcoholic extractive values suggested that the plant contained more polar compounds. The presence of phenolic chemicals and tannins in this plant part supports chemically the anti-diarrhoeal and antibacterial activities, which is consistent with previous studies [23].

Table 1: Physiochemical Parameters of *Mangifera indica* Seed Kernel

S. No	Parameter	Results
1	Foreign matter	Nil
2	Dry Powder Particle size	1.37µm
3	Wet Powder Particle size	2.2µm
4	Swelling index	0.5mL
5	Acid insoluble ash	2.8%
6	Water soluble ash	3.7%
7	Total ash	7.2%
8	Alcohol extractive	24%
9	Water extractive	25.9%

Fluorescence analysis is also a useful tool for determining the chemical composition of plant materials and for determining the ingredients in herbal drugs. The powder drug analysis was done by treating the samples with various chemical reagents and seeing them with visible and UV light of short and long wavelengths. The powdered seed kernel of *M. indica* were brown, yellow, black, yellowish green and green in colour (Table. 2). This demonstrated that the MISK has a variety of chromophores. The study indicates that the seed kernel contains phenols, alkaloids, terpenoids, flavonoids, tannins, lignins, saponins, and carbohydrates.

Table 2: Fluorescence Analysis of *Mangifera indica* Seed Kernel Powder

S. No	Test	0 hours		24 hours		48 hours	
		Day light	UV light	Day light	UV light	Day light	UV light
1	Plant Powder + Chloroform	Brown	Light brown	Brown	Greenish Brown	Brown	Green
2	Plant Powder + Hexane	Pale brown	Yellow	Pale brown	Pale brown	Light brown	Pale brown
3	Plant Powder + Benzene	Reddish brown	Brown	Reddish brown	Green	Reddish brown	Brown
4	Plant Powder + Aqueous NaOH	Reddish brown	Yellowish brown	Reddish brown	Yellowish brown	Reddish brown	Reddish brown
5	Plant Powder + alcoholic NaOH	Greenish yellow	Yellow	Greenish yellow	Green	Yellowish brown	Green
6	Plant Powder + 1N HCl	Light brown	Yellow	Light brown	Light brown	Light brown	Yellow
7	Plant Powder + Ethanol	Light brown	Greenish yellow	Light brown	Pale yellow	Light brown	Greenish yellow
8	Plant Powder + Ethyl acetate	Reddish black	Black	Black	Black	Black	Black
9	Plant Powder + Acetone	Light brown	Yellow	Light brown	Pale green	Light brown	Greenish yellow
10	Plant Powder + 50% H_2So_4	Meroon	Black	Black	Black	Black	Black

Table.3 shows the quality of the seed kernel's microbiological analysis. A microbiological quality examination was performed on the extracts using conventional procedures. The study examined the total number of bacteria, fungi, and pathogens. In the seed kernel, there were no enteric pathogens found. The total aerobic microbial load is within Ayurvedic and international pharmacopoeial guidelines.

Table 3: Microbial limit assay of *Mangifera indica* seed kernel

S. No	Test organism	Microbial counts CFU/g
1	Total aerobic Bacteria	$39x10^2$
2	Total Fungal count	$4x10^1$
3	Total Enteric Bacteria	Nil
4	Total *E. coli*	Nil
5	*Salmonella*	Nil

Synthetic drug use has become controversial as a response of the emergence of multidrug - resistant in clinical strains. In today's society, a new drug with minimal side effects that is also effective against MDR infections is required. Five bacteria were obtained from clinical samples and identified to evaluate the antibacterial properties of MISK aqueous and phenolic extracts. Identified isolates were considered and utilized for antimicrobial assay. Five clinical isolates such as *Eschericia coli, Salomonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* were identified based on growth pattern, microscopic nature and biochemical parameters [24]. Incidence of these organisms may vary depends on different biographic, geographic and socioeconomic status of the country.

Table 4: Antibacterial activity of *Mangifera indica* seed kernel aqueous extract (MISKAE)

S. No	Clinical isolates	Positive control (mm)	Negative control (mm)	Concentration in µg/Disc			
				200	400	600	800
1.	*Escherichia coli*	21±0.1	-	13±0.12	15±0.11	17±0.1	19±0.15
2.	*Salmonella typhi*	18±0.3	-	-	12±0.11	14±0.12	17±0.12
3.	*Staphylococcus aureus*	27±0.1	-	12±0.11	15±0.13	15±0.11	18±0.11
4.	*Pseudomonas aeruginosa*	22±0.2	-	10±0.12	12±0.11	13±0.11	14±0.11
5	*Streptococcus pyogenes*	20±0.1	-	-	12±0.12	13±0.13	14±0.13

MISKPE showed excellent antimicrobial activity at 400µg/ disc concentrations itself which was better than aqueous extract (Table 4). It produced 18±0.1, 15±0.11, 17±0.11, 18±0.11and 17±0.14 mm zone of inhibitions against *E.coli, Salmonella, Staphylococcus, Pseudomonas* and *Streptococcus* species. Good antimicrobial activity was exhibited by the extracts of MISK (Table 5)

Table 5: Antibacterial activity of *Mangifera indica* seed kernel phenolic extract (MISKPE)

S. No	Clinical isolates	Positive control (mm)	Negative control (mm)	Concentration in µg/Disc			
				50	100	200	400
1	*Escherichia coli*	21±0.1	-	14±0.1	16±0.12	17±0.12	18±0.1
2	*Salmonella typhi*	18±0.3	-	-	11±0.11	13±0.12	15±0.11
3	*Staphylococcus aureus*	27±0.1	-	12±0.11	15±0.12	16±0.13	17±0.11
4	*Pseudomonas aeruginosa*	22±0.2	-	10±0.11	12±0.13	15±0.12	18±0.11
5	*Streptococcus pyogenes*	20±0.1	-	-	12±0.11	14±0.12	17±0.14

MISKPE produced antibacterial activity at a concentration of 50 µg/ against *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa*. MISKPE showed better antimicrobial activity than MISKAE. At 200 µg/disc concentration MISKAE didn't showed any antimicrobial activity against *Salmonella typhi* and *Streptococcus pyogenes* where as MISKPE showed good antimicrobial activity. At a concentration of 200µg the extract produced a zone of inhibition of at least 10mm against all of the species tested. The MIC value, in simple terms, represents the ability of each extract to suppress microbial growth at the lowest concentration. The strains with high positive results against the tested *M.indica* extracts were chosen for further studies to estimate the MIC value, and the MIC value is provided in Table 6. According to these findings, there are significant differences in the MIC of both aqueous and phenolic seed kernel extracts. The majority of antibacterial components discovered in plants are aromatic or saturated chemical compounds that are more soluble in polar solvents like water and organic solvents. Aqueous extracts, is from the other side, were less effective. This is due to the existence of aqueous -soluble chemicals such as polysaccharides and polypeptides, which are often, used as pathogen adsorption inhibitors but have no effect as antibacterial agents [25]. The antibacterial activity of aqueous extracts gives scientific support for the use of aqueous extracts in traditional illness therapy. There is further proof to show that an organic solvent is a better solvent for extracting antibacterial compounds from medicinal plants consistently. A drug dilution method was followed to measure the minimal inhibitory concentration of MISKAE and MISKPE extracts. Bacterial growth inhibition studies were assessed exclusively by the way of expressing MIC. MISKPE showed efficient bacterial inhibition at lower doses when compared to aqueous extract (Table 6). MISKPE inhibited *E. coli* at a 122 µg/ml concentration, whereas it was only at 170 µg/ml by MISKAE. Plant extracts tested in this work revealed good antimicrobial activity. The highest extracts created more zone of inhibition against different pathogen while compared to the positive control. This would recommend that plant drug sources may be considered the best replacement for recent antibiotic therapy.

Table 6: Minimal inhibitory concentration of *Mangifera indica* aqueous and phenolic extract.

S. No	Clinical isolates	MIC value of µg/ml	
		Aqueous extracts	Phenolic extract
1	*Escherichia coli*	170	122
2	*Salmonella typhi*	225	175
3	*Staphylococcus aureus*	250	175

4	*Pseudomonas aeruginosa*	275	200
5	*Streptococcus pyogenes*	325	250

Terpenoids, flavonoids, saponins, phenolic compounds, tannins, lignin, protein, and total carbohydrates were found in the aqueous extract, whereas alkaloids, terpenoids, flavonids, phenolic compounds, and tannins were found in the phenolic extract. This test is necessary to determine the primary nature of the phytochemicals present in the extract. The biological activity of phenolic substances, including flavonoids, terpenoids, and tannins, was revealed in this study. (Table 7).

Table 7: Phytochemical analysis of *Mangifera indica* seed kernel

S. No	Test	Reagent	Observation	Aqueous extract	Phenolic extract
1	Alkaloids	Mayer's	Creamy white precipitate	Negative	Positive
2	Steroids	Acetic anhydride	Reddish brown precipitate	Negative	Negative
3	Terpenoids	Chloroform	Reddish brown precipitate	Positive	Positive
4	Flavonoids	Ethyl Acetate Test	Yellow colour	Negative	Positive
5	Saponins	Extract + boiled	Foam formed	Positive	Positive
6	Tannins	Ferric Chloride Test	Brownish green of Blue Black	Positive	Positive
7	Lignin	Safranin	Pink colour	Positive	Negative
8	Phlobatannins	Hcl	Red precipitate	Negative	Negative
9	Fat and Oil	Copper sulphate + sodium hydroxide	Blue Colour	Negative	Negative
10	Test for Glycosides	Potassium hydroxide	Red colour	Positive	Positive
11	Inulin	I-napthol	Brownish red	Negative	Negative
12	Anthroquinones	H_2SO_4 +Chloroform+ ammonia	No colour change	Negative	Negative
13	Cardiac glycosides	Glacial acetic acid	Interface brown ring	Positive	Positive
14	Proteins	Xanthoprotein	Orange colour	Positive	Positive
15	Carbohydrates	Barfoed's reagent	Reddish brown	Positive	Positive
16	Amino acids	Ninhydrin	Violet colour	Positive	Positive
17	Phenolic compounds	Alcohol + Ferric Chloride	Bluish green	Positive	Positive

Standard spectrophotometric methods were employed to measure significant phenolic compounds in the extracts and the results showed that the aqueous extract yielded 2.6% tannins, 44% phenolic compounds and 1.8% of flavonoids. Phenolic extracts of MISK had a higher content of certain secondary metabolites, such as flavonoids (6.2%), tannins (14%), and phenols (49.5%) (table 8). Terpenoids, tannins, phenolic compounds, and flavonoids also confirm the existence of a wide range of phytochemicals.

Table 8: Quantitative phytochemical analysis of *Mangifera indica* seed kernel.

S. No	Phytoconstituents	% Yield (water)	% Yield (Phenolic)
1	Flavonoids	1.8±0.35	6.2±0.45
2	Tannins	2.6±0.23	14.±0.62
3	Polyphenols	44±0.81	49.5±0.76

Tannins have been linked to a wide range of antimicrobial properties [26]. Some researchers have discovered that phenols that have been substantially oxidised are more inhibitory [27]. Plants produce flavonoids it active against to microbial infection [28]. Terpenoids are antibacterial (antifungal and antiprotozoal [29]. As a result, the plant that was studied revealed the presence of active phytochemicals that have a variety of beneficial qualities.

Conclusion

Using an ayurvedic and herbal approach, the plant is beneficial in the treatment of a variety of disorders. The ethnomedicinal study of plants is important for modern medicine, but its utility will be restricted unless processes are standardised to achieve consistent and repeatable results. *Mangifera indica* seed kernel is one of the most significant plant part traditionally used for the treatment of gastrointestinal infections. MISKAE and MISKPE were subjected for biopotential study to screen antibacterial activity. Very good antibacterial activity was exhibited by both the extracts at 200 - 400µg concentration against multidrug resistant gram positive as well as gram negative clinical isolates. *M. indica* seed kenel are considered to be a potential source of bioactive compounds. The results of the qualitative analysis showed that tannin, sapanoin, flavonoids, terpenoids, alkaloids, carbohydrate, polyphenols and glycoside present in MISKAE and MISKPE. The established specifications ensure that the quality of the crude drug is retained and could also be used to develop a monograph.

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