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AN OVER VIEW ON *MOMORDICA DIOICA ROXB* SEEDS IT'S PHYTOCHEMICAL, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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ABSTRACT
Momordica dioica Roxb., also referred to as teasel gourd or spiny gourd, is a medicinal plant that is widely employed in Asian traditional medical systems. Although its fruits and roots have received a lot of attention, new research has shown how important its seeds are as a source of bioactive substances. An extensive summary of the phytochemical makeup and antioxidant capacity of M. dioica seeds is provided in this review. Its therapeutic potential has been enhanced by the discovery of flavonoids, phenolic acids, saponins, alkaloids, and essential fatty acids through phytochemical screening. . DPPH, ABTS, and FRAP are just a few of the in vitro antioxidant tests that have shown the seeds' ability to scavenge free radicals and lessen oxidative stress. These antioxidant qualities point to possible uses in the creation of pharmaceutical formulations and natural health supplements. To confirm these characteristics and clarify the underlying mechanisms of action, more research is necessary, including in vivo and clinical trials.

KEYWORDS: Herbal extract, Extraction Methods, Phytochemical activity, Antimicrobial study, Antioxidant activity.

INRODUCTION

Momordica dioica is a climbing creeper that is dioecious and a member of the Cucurbitaceae family. Common names for it include teasle gourd, spine gourd, kantola, karola, kantroli, kantoli, and kakrol. According to the most recent edition of Indiam *Momordica*, there are six species that have been identified, four of which are dioecious and two of which are monoecious. India, Bangladesh, Sri Lanka, Myanmar, China, Japan, South East Asia, Polynesia, Tropical Africa, and South America are among the places where it can be found growing. A favourite summer food, the plant grows to a length of 5 to 7 meters, and its leaves, fruits, and young twigs are utilised as vegetables.

The plant Momordica dioica contains a variety of phyto constituents, including alkaloids, steroids, triterpenoids, flavonoids, glycosides, ursolic acid, vitamins, minerals, and fibre. It is also frequently used in cooking and has been traditionally used to treat diabetes, liver disorders, intestinal worms, infestations, skin diseases, etc.[2]

Fig. No. 1: *Momordica Dioica* Fruit.

Momordicadica dioica is a type of vine that bears fruit from November to September and flowers from June to July. Because the male and female flowers are born apart, the plant is monosexual. Both male and female nodes produce flower buds. Beginning in the second week of August and continuing until the first week of October, male buds are generated. From the first to the second week of September until the third week of October, flowers will bloom. Female flowes have three nector glands and are tiny and yellow in appearance. Male flowers are pale yellow in hue and are length around 2.8cm.

The plant's exocarp is delicate, and its fuits have short beaks. Because of the firm endocarp, it exhibits tolerance against knot nematodes, caterpillars, and gall flies. Simple, widely oval leaves with deep lobes in the outline are 3.8–10 cm in length. With their hepatoprotective, laxative, diuretic, and antivenom qualities, fruits can treat a variety of conditions, including asthma, digestive and mental disorders, lizard-induced inflammation, excessive salivation, etc. They exhibit allelopathic and antioxidant properties.[3]

This can draw attention to the high nutritional content of wild edible plants that people in rural areas regularly or occasionally eat. It has been observed that when food is in short supply due to drought, floods, or other calamities, these civilisations resort to consuming a variety of herbal treats. The nutritional value of many edible wild plants was used in emergency situations.

Fig. No. 2: Momordica Dioica Flowers.

Fruits are the juicy seed-bearing structures of blooming plants that can be eaten. A low-protein diet is consumed by many people in both urban and rural settings, raising the risk of malnutrition and nutritional diseases. Children are especially vulnerable, especially those who are pregnant or nursing. In developing nations, a wide variety of edible wild plants are employed as food sources to provide additional nutrients to the local populace. In developing nations, a wide variety of edible wild plants are utilised as food sources to provide additional nutrition to the local populace. The plant has simple, membrane-based, roughly oval leaves that range in length from 3.8 to 10 cm to 3.2 to 8 cm. The leaves are pierced, glabrous, glandular, and petiole 1.3 to 4.5 cm long. The base of the leaves is cordate, and the leaves are deeply lobed in three to five triangular lobes. The leaves of the plant contain antihelminthic and aphrodisiac qualities. It is also used to treat liver damage, fever, bronchitis, asthma, bowel affection, jaundice, piles, mental digestive disorders, and urinary complaints. It also balances tridosha and alters pitta. The leaf juice is mixed with coconut, pepper, red sandalwood, and other substances to make a topically applied salve that is used to treat headaches.

Fig. No. 3: Mommordica Dioica Fruit

To cure skin disorders, fruit powder can be taken orally twice or three times a day, and leaf paste can be applied externally. Cucumbers contain this type of fruit, which is fleshy. Vascular bundles are present on both sides of most members. The edible fruit of M. dioica was found to have an average nutritional value of 84.1% moisture, 7.7% carbohydrates, 3.1% protein, 3.1% fat, 3.0% fibre, and 1.1% minerals.

Fresh fruit juice is advised for hypertension. The fruit can be fried in a small bit of oil. It also contained trace levels of important vitamins such as ascorbic acid, riboflavin, thiamine, carotene, and niacin.[4,5] The fruit has a short beak, is obtuse with a red kernel within, is thickly echinate, has soft spines, and matures to green and yellow. Green fruits are typically utilised as vegetables. It has many medicinal advantages. Hepatoprotective, stomachic, laxative, diuretic, alexiteric, and antivenum qualities are all found in fruits. It is also used to treat snake bites, elephantiasis, leprosy, asthma, and excessive salivation. used to treat fever, mental disorders, digestive problems, and mucous membrane discharge. It can also be consumed to treat diabetes. Tender fruits are applied to the skin to treat acne and pimples.[5] These plants have been utilised for many different purposes, but not enough research has been done on their nutritional content, bioavailability, and suitability as dietary supplements for significant nutritional and anti-nutritional features.[6] M. dioica was chosen since it is a common vegetable consumed by the locals and is used for a variety of purposes in rural areas. Additionally, a number of research findings highlight M. Dioica's potential. Hepatoprotective, stomachic, laxative, diuretic, alexiteric, and antivenum qualities are all found in fruits. It is also used to treat snake bites, elephantiasis, leprosy, asthma, and excessive salivation. used to treat fever, mental disorders, digestive problems, and mucous membrane discharge. It can also be consumed to treat diabetes. Tender fruits are applied to the skin to treat acne and pimples.[5] Sweet gourds are used as a colourant for red glutinous rice, also called xoi gac, in which the aril and seeds are boiled in glutinous rice to add colour and flavour. In Thailand and India, however, tender fruits are consumed as vegetables. The seed membranes are used as a medicinal herb in Vietnam to treat dry eyes and encourage healthy vision. Similar to this, traditional Chinese medicine uses the seeds of the sweet gourd, known as mubiezi, for a variety of internal and external purposes. Spine gourds are ripe, mature, and tender fruits that are deseeded, cooked like vegetables, roasted, and then made into chutney with seasonings and coconut. Ripe fruits are consumed raw, while the aril of mature seeds is consumed as a beverage. In addition to its use as a vegetable, it is valued for several medicinal and restorative properties. Unlike other species, M. suban gulata subsp. renigera appears to have no multiple ethnobotanic importance and is cultivated commercially for its early fruits and branches. Mostly farmed by poor agricultural communities who rely on them for their livelihood, these crops are only cultivated in certain geographic locations within different agro-ecological zones. These plants have been utilised for many different purposes, but not enough research has been done on their nutritional content, bioavailability, and suitability as dietary supplements for significant nutritional and anti-nutritional features.[6] M. dioica was chosen since it is a common vegetable consumed by the locals and is used for a variety of purposes in rural areas. Unlike other species, M. suban gulata subsp. renigera appears to have no multiple ethnobotanic importance and is cultivated commercially for its early fruits and branches. Mostly farmed by poor agricultural communities who rely on them for their livelihood, these crops are only cultivated in certain geographic locations within different agro-ecological zones. These plants have been utilised for many different purposes, but not enough research has been done on their nutritional content, bioavailability, and suitability as dietary supplements for significant nutritional and anti-nutritional features.[6] *M. dioica* was chosen since it is a common vegetable consumed by the locals and is used for a variety of purposes in rural areas. Additionally, a number of research findings highlight M. Dioica's potential from a variety of angles, including plant parts, animal models, and conventional medications.

Fig No. 4: *Momordica dioica* seeds.

June to July is when flowers bloom, and September to November is when fruit ripens. Simple membranous, roughly oval in outline, 3.8-10 cm by 3.2-8 cm in length, cordate at the base, deeply lobed in 3-5 triangular lobes, punctated, whole but distantly denticulate, petiole 1.3-4.5 cm long, channelled above, pubescent, and glandular are the characteristics of the plant's leaves. Male bloom is solitary, up to 2.8 cm long and golden coloured. Petals are oblong and lanceolate, measuring 1.3 to 2.5 cm. Calyx: linear lanceolate, five lobed. Three stamens and five partite corollas. As in males, females have a single, tiny bract beneath the centre of the peduncle, calyx, and corolla, while males have three united glands, an ovary covered in long, soft papillae, and an ellipsoid with many ovules. The colour is yellow. The fruit has a short beak, is obtuse with a red kernel within, is thickly echinate, has soft spines, and matures to green and yellow. Encased in scarlet pulp, the seeds are spherical, broadly ellipsoid, somewhat compressed, and faintly and irregularly corrugated. glabrous, shiny, wrinkled, branching, and slender stem. Tendrils are glabrous, elongated, simple, and striate.[1–6] This climbing creeper is typically found in Bangladesh, India, Pakistan, the Himalayas, and Ceylon. reported as high as 1500 meters in the Meghalayan Garo Hills and Assam.[7] The Indo-Malayan region is where the Cucurbitaceous crop kakrol first appeared.

Grouping[16] The PLANTS Database. The Kingdom Subkingdom Tracheobionata Plantae Super division Division of Spermatophyta Order of Magnoliphyta Class Subclass Family Magnoliopsida Violales Cucurbitaceae Dilleniidae Momordica Genus Dioica species.

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Synonyms International Journal of Phytomedicine 2 (2010) 01-09 Bawara et al. Kartoli, small bitter gourd, spine gourd, Kakora, Parora, Golbandra, Bengoli, Hindi, and English Tamil: Aegaravalli, Tholoopavai, Paluppakkay; Marathi: Kartoli; Malyalam: Venpaval, Erima Pasel Karkotaki, Madahagala, Agakara Cannad, Vahisi Panjabi, Dharkarela Assam, and Batkarila classifications[16] The PLANTS Database. The Kingdom Subkingdom Plantae Super division Tracheobionata Magnoliphyta Class Subclass Order Family Magnoliopsida Division Spermatophyta Violales Cucurbitaceae Dilleniidae Species dioica of the Genus *Momordica*.

Plantae, Plantae, Plants, Vegetal Subkingdom: Tracheobionta, or vascular plants, is the taxonomic hierarchy. Division: Magnoliophyta, which includes flowering plants, phanérogames, angiosperms, Magnoliopsida is the class that includes dicots, dicotylédones, and dicotyledons. Subclass, Order, Family, Species, and (a) Citrouilles, gourdes, squashes, and Dilleniidae Violales Cucurbitaceae Roxb. ex. Willd. momordica dioica.

Plant parts: Fruits: Green fruits are typically utilised as vegetables. It has numerous therapeutic benefits. Fruits have hepatoprotective, stomachic laxative, diuretic, and antivenum properties. Asthma, leprosy, excessive salivation, lizard and snake bite irritation, elephantiasis, fever, mental health issues, digestive issues, heart problems, and mucous membrane discharge are among its other uses.[3, 10]

Fig. No. 5: ***Momordica dioica.***

For hypertension, fresh fruit juice is recommended. The fruit is used to cure diabetes after being cooked in a modest amount of oil. Acne and pimples are treated by rubbing tender fruits over the skin. To treat eczema, seeds are roasted and consumed. and other skin issues.[11] When applied to the nostrils, the powder or infusion of the dried fruits causes a strong errhine effect and causes the Schneiderian mucous membrane to discharge copiously. The plant's leaves have aphrodisiac and antihelminthic properties. Tridosha, fever, pitta, jaundice, asthma, bronchitis, piles, hepatic damage, mental digestive issues, bleeding piles, bowel affection, and urinary complaints are among the other conditions it is used to treat. To cure head ache, the leaf juice is combined with coconut, pepper, red sandalwood, and other ingredients to create an ointment that is used topically. For skin conditions, apply leaf paste topically and take it orally two or three times a day.

Roots: Momordica dioica roots have many therapeutic benefits. Root juice has antibacterial, astringent, and stimulating properties. The mucilaginous tubers are used to treat bleeding piles, related intestinal disorders, and urinary problems. They are also antihelmintic, spermicidal, and antifertility abortificant.[13] Root powder is applied to the skin to soften it and lessen sweating. Female plants' mucilaginous tubers are used to treat intestinal infections and bleeding piles; two drachmas or more should be taken twice a day. To overcome diabetes, 50ml of the juice from a tuberous root mashed in hot water is taken orally once day on an empty stomach for two days. The toasted root is used to treat digestive issues and to halt bleeding from piles. In the Konkan area, the root's juice is used as a home remedy for inflammation brought on by coming into contact with the house lizard's urine. The male creeper's root is used to treat ulcers, particularly those brought on by snake bites, and its roots are also suggested for scorpion stings.[3] When ground into a paste and applied all over the body, the root is thought to have a sedative effect on high fever and delirium.

NUTRITIONAL VALUES OF *MOMORDICA DIOICA*

Momordica dioica's nutritional values (per 100 grammes, approximately):

Amount of Nutrients
17–25 kcal of energy
The carbohydrate 3–4 g of protein 2-3 g
0.2–0.5 g of fat
Fibre in the Diet 1.5–3 grammes
25–40 mg of vitamin C
β-carotene, or vitamin A 100–300 µg
Calcium 25–50 mg
1-2 milligrammes of iron
200–250 milligrammes of potassium
About 30 milligrammes of magnesium
About 30 milligrammes of phosphorus

Table No. 1: Nutritional Values.

Vitamins (g/100g)	*M. dioica*	*Recommended dietary allowances (mg/day)**
Vitamin A	2.5	-
VitaminB1 (Thiamine)	1.8	1.7
Vitamin B2 (Riboflavin)	3.5	1.7
Vitamin B3 (Niacin)	1.9	18
Vitamin B5 (Pantothenic Acid)	18	-
Vitamin B6(Pyridoxine)	4.3	2.0
Vitamin B9 (Folic Acid)	3.6	0.2
Vitamin B12 (Cyanocobalamin)	4	0.001
Vitamin C (Ascorbic Acid)	-	40
Vitamin D2 & 3 (Cholecalciferol)	3	-
Vitamin H (Biotin) g/100g	6.5	-
Vitamin K (Phytonadione)	15	-

Advantages for Health

• Packed with antioxidants: Flavonoids and phenolic compounds are present.
• Anti-diabetic: Used historically to reduce blood sugar levels.
• Digestive aid: A diet rich in fibre promotes intestinal health.
• Anti-inflammatory: Used to lessen inflammation in Ayurvedic therapy.

Advantages for Health

In addition to being nutrient-dense, spiny gourd has other health advantages.

- Blood Sugar Regulation: Compounds in spiny gourd may help control blood sugar levels, which is advantageous for diabetics.
- Antioxidant Properties: Packed with antioxidants, it fights oxidative stress and may lower the chance of developing chronic illnesses.

- Controlling weight: By encouraging satiety, spiny gourd, which is low in calories and high in fibre, can help with weight management.
- Digestive Health: Its high fibre content helps ease constipation and promotes a healthy digestive system.
- Skin Health: Vitamins A and C help maintain healthy skin, which may lessen ageing symptoms and encourage a clear complexion.
- Liver Detoxification: Spiny gourd may help the body rid itself of toxins by supporting liver function and detoxification procedures.
- Support for the Immune System: The vitamins and minerals in spiny gourd can strengthen the body's defences against diseases and infections.
- Anti-inflammatory Effects: Its anti-inflammatory properties may reduce inflammation, benefiting conditions like arthritis

Culinary Uses: Spiny gourd is a versatile ingredient that can be prepared in a variety of ways. It can be added to vegetable curries or stews to add texture and nutrition. It can also be sliced and stir-fried with other vegetables and spices for a quick and healthful meal. Its distinct flavour can be used to make pickles, which give meals a tangy twist. Finally, it can be blanched and added to salads to add a crunchy element.

Nutritional Composition (per 100g, dry weight) According to a study published in the Journal of Food and Pharmaceutical Sciences, the nutritional composition of spiny gourd is as follows: • Moisture: 87% • Ash: 14% • Crude Protein: 52.06% • Crude Fiber: 15.36% • Crude Fat: 4% • Carbohydrates: 14.58% Vitamins (mg/100g dry weight): • Vitamin A: 2.5 • Vitamin B1 (Thiamine): 1.8 • Vitamin B2 (Riboflavin): 3.5 • Vitamin B6: 4.3 • Vitamin H (Biotin): 6.5 • Vitamin K: 1 Minerals (mg/100g dry weight): • Potassium: 370 • Sodium: 58 • Calcium: 26,000 • Zinc: 8.5 • Copper: 1.7 • Magnesium: 14,000 Fatty Acids (% of total fat): • Oleic Acid: 56.25% • Palmitic Acid: 12.16% • Linoleic Acid: 22.51% • Myristic Acid: 3.59% • Stearic Acid: 3.55%

EXTRACTION METHODS

1. Extraction: The shade dried plant material was pulverised into a coarse powder and extracted in soxhlet apparatus using hexane as solvent. After being collected, the hexane extract was vacuum-distilled and weighed. After that, the marc was extracted using methanol as described above. The dried methanol extract was suspended in water and fractionated with ethyl acetate. The soluble portion of ethyl acetate was separated, concentrated under vacuum distillation and then weighed.[14] These fractions were then subjected for the qualitative phytochemical analysis.

2. One sample was taken from the *Momordica dioica* plant's field in the surrounding district. The roots were cleaned by washing them thoroughly two or three times with running tap water and once with sterile distilled water to get rid of any impurities. They were then chopped into small pieces and allowed to dry in the shade. Finally, they were coarsely powdered separately and kept in tightly sealed containers for future use in the lab.

3. Maceration

Description: Plant material is soaked in a solvent for a prolonged period, often at room temperature or slightly warm circumstances. Although it is used to extract sensitive chemicals, it is less effective than other techniques. The procedure involves chopping or crushing plant material and then soaking it in a solvent. o The extract is obtained by filtering the liquid after a predetermined amount of time. Applications: Herbal infusions, oils, and tinctures. Examples include tinctures and herbal teas prepared from plants like echinacea.

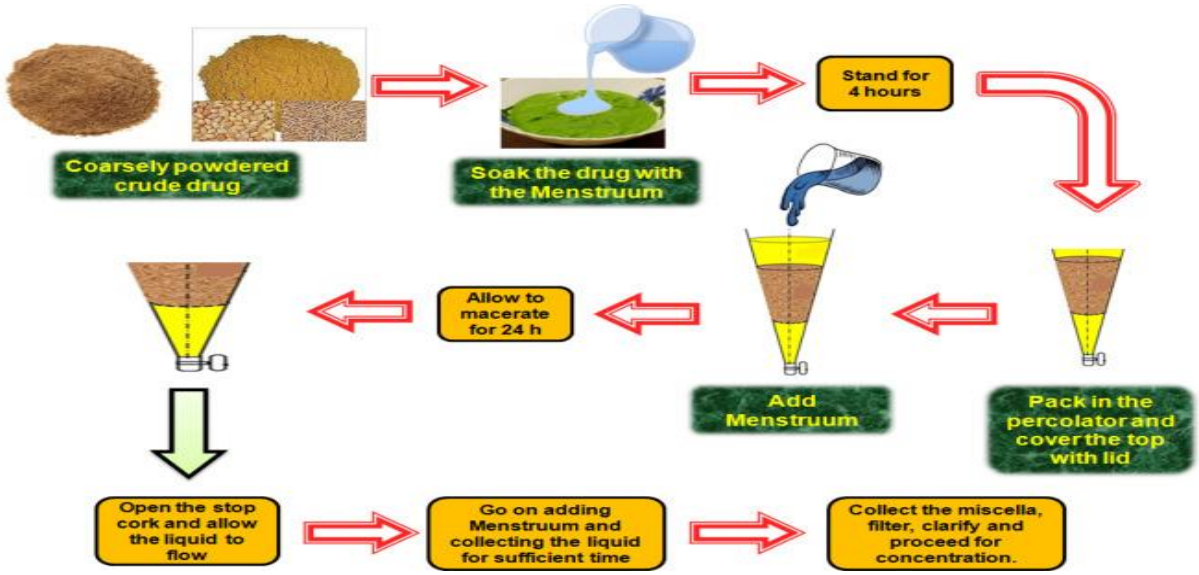

Fig. No. 6: Maceration.

4. Alcoholic Extraction (Ethanol Extraction)
Description: A variety of chemicals are extracted from plant material using alcohol, often ethanol, particularly for the production of tinctures or medicinal extracts.

The procedure involves soaking plant material in ethanol.

The extract is filtered after a predetermined amount of time, and the alcohol is removed to reveal the required components.

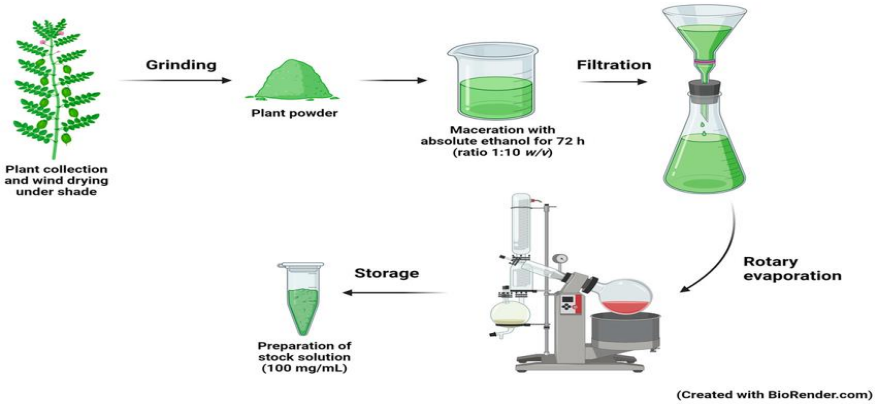

Fig. No. 7: Alcoholic Extraction.

5. Decoration Extraction
The process of locating and removing ornamental features from fabric or objects, such as textiles, needlework, or craftwork, is known as decoration extraction.
1. Image Preprocessing: o Convert to Greyscale: To make processing easier, convert the image to greyscale if colour information is not required.

Noise Reduction: To eliminate noise and sharpen edges, use filters like the Median Filter or Gaussian Blur.

Edge Detection: To find boundaries in an image that create decorative features, use edge detection techniques such as Canny Edge Detection.

2. Segmentation: o Thresholding: Use thresholding to assist isolate the decoration by distinguishing it from the backdrop.

o Region Growing or Watershed: To further isolate distinct elements, apply algorithms such as region growing or watershed segmentation.

3. Feature Extraction
To find certain ornamental patterns, extract important characteristics (edges, textures, forms, and colours) using methods like SIFT (Scale-Invariant Feature Transform) or Histogram of Orientated Gradients (HOG).

If you're searching for particular themes or patterns in the image, use template matching.

4. Post-Processing
Morphological Operations: To hone the retrieved features and eliminate extraneous details, apply dilation, erosion, and opening/closing operations.

Object identification: If necessary, identify particular decorative features (such as floral patterns in textiles or furniture accessories) using object identification algorithms (such as YOLO or SSD).

5. The last extraction
For later usage (such as for textile printing, design inspiration, etc.), separate and store the extracted decorations as separate elements or as a component of a design system.

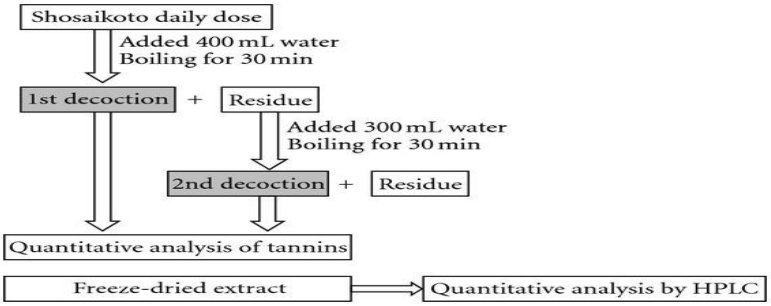

Fig. No. 8: Decoration Extraction.

PHYTOCHEMICAL ACTIVITY

Preliminary Qualitative Analysis

a. The Mayer test Two drops of Mayer's reagent are applied along the test tube's sidewalls to a few millilitres of plant sample extract. Alkaloids are present when a white, creamy precipitate appears.

a. The Wagner test Along the test tube's sides, a few drops of Wagner's reagent are added to a few millilitres of plant extract. A reddish-brown precipitate indicates a positive test result.

2. Test for Amino acids

After dissolving 100 mg of the extract in 10 millilitres of distilled water, the filtrate is run through Whatmann No. 1 filter paper and tested for amino acids.

a. Test for ninhydrin Two millilitres of aqueous filtrate are mixed with two drops of ninhydrin solution (10 mg of ninhydrin in 200 millilitres of acetone). The presence of amino acids is indicated by a purple appearance.

3. Do a Carbohydrate Test

a. The Molish test Two drops of α-naphthol alcoholic solution are added to two millilitres of plant sample extract. After giving the mixture a good shake, a few drops of strong sulphuric acid are gradually added along the test tube's sides. Carbohydrates are indicated by a violet ring.

b. Benedict's examination Benedict's reagent (0.5 ml) is added to 0.5 ml of filtrate. For two minutes, the mixture is heated in a boiling water bath. The presence of sugar is indicated by a distinctively coloured precipitate

4. Check for Fats and Fixed Oils

a. The spot test A tiny amount of extract is sandwiched between two filter sheets. The presence of fixed oils is indicated by an oil stain on the paper.

b. Test for saponification A small amount of extract is mixed with a drop of phenolphthalein and a few drops of 0.5 N alcoholic potassium hydroxide solution. For two hours, the mixture is heated in a water bath. The presence of fixed oils and fats is indicated by the formation of soap or the partial neutralisation of alkali.

5. Test for Glycosides

After hydrolysing 50 mg of extract for two hours in a water bath with strong hydrochloric acid, the hydrolysate is filtered and put through the following tests.

b. Legal’s test

After dissolving 50 mg of extract in pyridine, 10% NaOH is added to create an alkaline solution. The colour pink indicates the presence of glycoside.

a.Test for Phenolic compounds and Tannin

a. Test for ferric chloride Five millilitres of distilled water are used to dissolve the extract (50 mg). A small amount of neutral 5% ferric chloride solution is added to this. Phenolic compounds are indicated by a dark green hue.

b. Gelatin test

50 mg of the extract is diluted in 5 ml of distilled water, and then 2 ml of a 1% gelatin solution with 10% NaCl is added. Phenolic chemicals are present when a white precipitate forms.

c. Lead acetate test

After dissolving the extract (50 mg) in distilled water, 3 millilitres of a 10% lead acetate solution are added. Phenolic chemicals are indicated by a large, white precipitate.

d. Alkaline reagent test

10% ammonium hydroxide solution is used to treat an extract aqueous solution. Flavonoids are indicated by yellow fluorescence.

e. Magnesium and Hydrochloric acid reduction

After dissolving the extract (50 mg) in 5 ml of alcohol, a few pieces of magnesium ribbon and droplets of strong hydrochloric acid are added. It is assumed that flavonol glucosides are present if any pink to scarlet colouration appears.

7. Test for phytosterols

a. Libermann-Burchard’s test

Two millilitres of acetic anhydride are used to dissolve the extract (50 mg). Along the test tube's sidewalls, one or two drops of strong sulphuric acid are gradually added to this. The presence of phytosterols is indicated by a variety of colour changes.

8. Test for Proteins

After dissolving the extract (100 mg) in 10 millilitres of distilled water and filtering it through Whatmann No. 1 filter paper, the filtrate is tested for proteins.

a. Millon’s test

A few drops of Millon's reagent are added to two millilitres of filtrate. When proteins are present, a white precipitate forms.

b. The Biuret test

One drop of a 2% copper sulphate solution is added to two millilitres of filtrate. One millilitre of 95% ethanol is added to this, and then an excess of potassium hydroxide pellets. The presence of protein is shown by a pink ethanolic layer.

9. Test for Saponins

Distilled water is used to dilute the extract (50 mg) to make 20 ml. For fifteen minutes, the suspension is shaken in a graded cylinder. The presence of saponins is indicated by a two-centimeter layer of foam.

10. Test for gum and Mucilages

After dissolving the extract (100 mg) in 10 millilitres of distilled water, 2 millilitres of 100% alcohol are added while being continuously stirred. The presence of gums and mucilages is indicated by white or hazy precipitation.

11. Test for volatile oil

50 mg of powdered material (a crude medication) is taken and hydro-distilled in order to estimate the volatile oil. The distillate is collected in the assembly's graduation tube, where the volatile oil and aqueous portion are automatically separated.

ANTIMICROBIAL ACTIVITY

1. DISC DIFFUTION/CUPPLATE METHOD

The bacteria samples were uniformly injected onto the surface of an agar plate using the agar well diffusion method with streptomycin and ampicillin. Using the spread plate technique, all of the bacteria samples were spread out on each plate. Then, wells were bored into the agar plate, and a standard quantity of an antibiotic was applied. The antibiotic was then allowed to diffuse into the adjustment medium. After that, these plates are incubated at 37°C for 24 hours.

Each plate was examined 24 hours later. Using the standard zone diameter scale (HIMEDIA), the diameter of the well as well as the zones of full inhibition were assessed. Standard tables are used to interpret the zones of inhibition's sizes (Johnson et al., 1995). Zones of growth inhibition are observed and noted in millimetres as a bacterial lawn develops on the plate. The antibiotic's diffusion rate, the microorganisms' level of sensitivity, and the bacterium's growth rate all affect the size of the zone of inhibition.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will prevent the organism from growing visibly. The "gold standard" for MIC determination is generally agreed to be doubling the dilution step up and down by 1 mg/ml as needed. Though the process merely yields interval-censored reading, the value is produced in a highly automated manner. According to the MIC principle, each antimicrobial agent is diluted on a log 2 scale to produce a range of concentrations. A standardised suspension of the microorganisms to be tested is placed in each well of the plate containing the antimicrobial agent. There is an inverse relationship between the zone diameter of inhibition and the MIC. In other words, the zone of inhibition is bigger and the MIC is lower the more susceptible the microbe is to the antimicrobial drug. On the other hand, the MIC and zone of inhibition decrease with increasing microorganism resistance.[5]

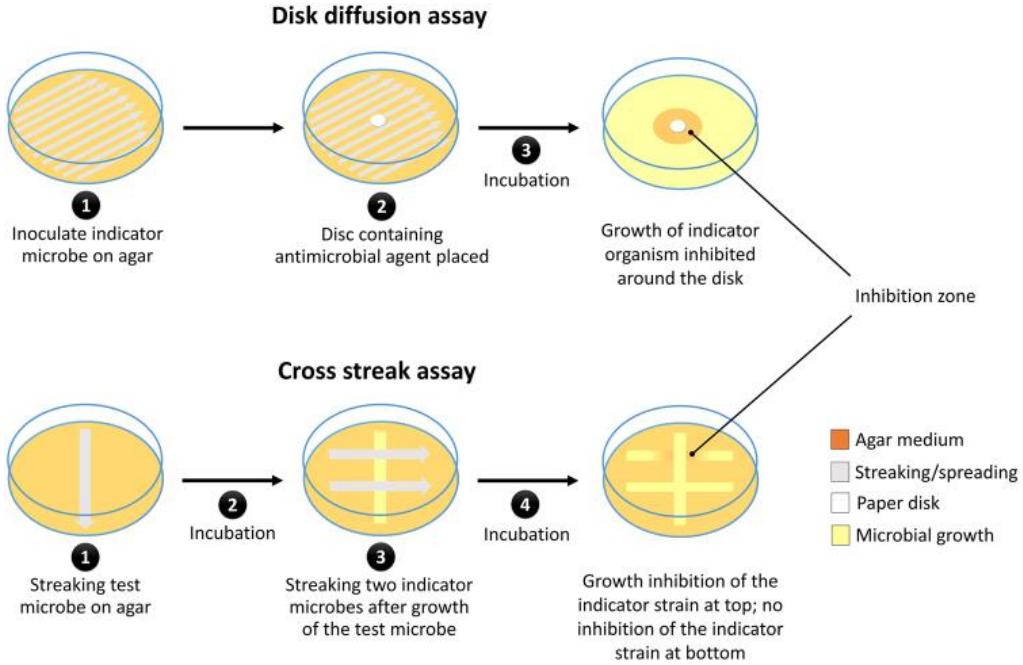

Fig. No. 9: Cup Plate Method.

2. AGAR DILUTION /BROTH DILUTION METHOD

The MIC of antimicrobial drugs can be found using two very comparable methods: the broth dilution method and the agar dilution. method. The minimum inhibitory concentration (MIC) is the concentration of an antimicrobial agent that prevents a certain bacterium from growing visibly. The medium used is the primary distinction between the broth and agar dilution techniques: While broth dilution uses liquid broth tubes, agar dilution uses agar plates. In both techniques, a standardised amount of microbial cells are applied to the medium after varying doses of the antimicrobial material have been added. By monitoring the microorganism's development or lack thereof, the MIC is ascertained.

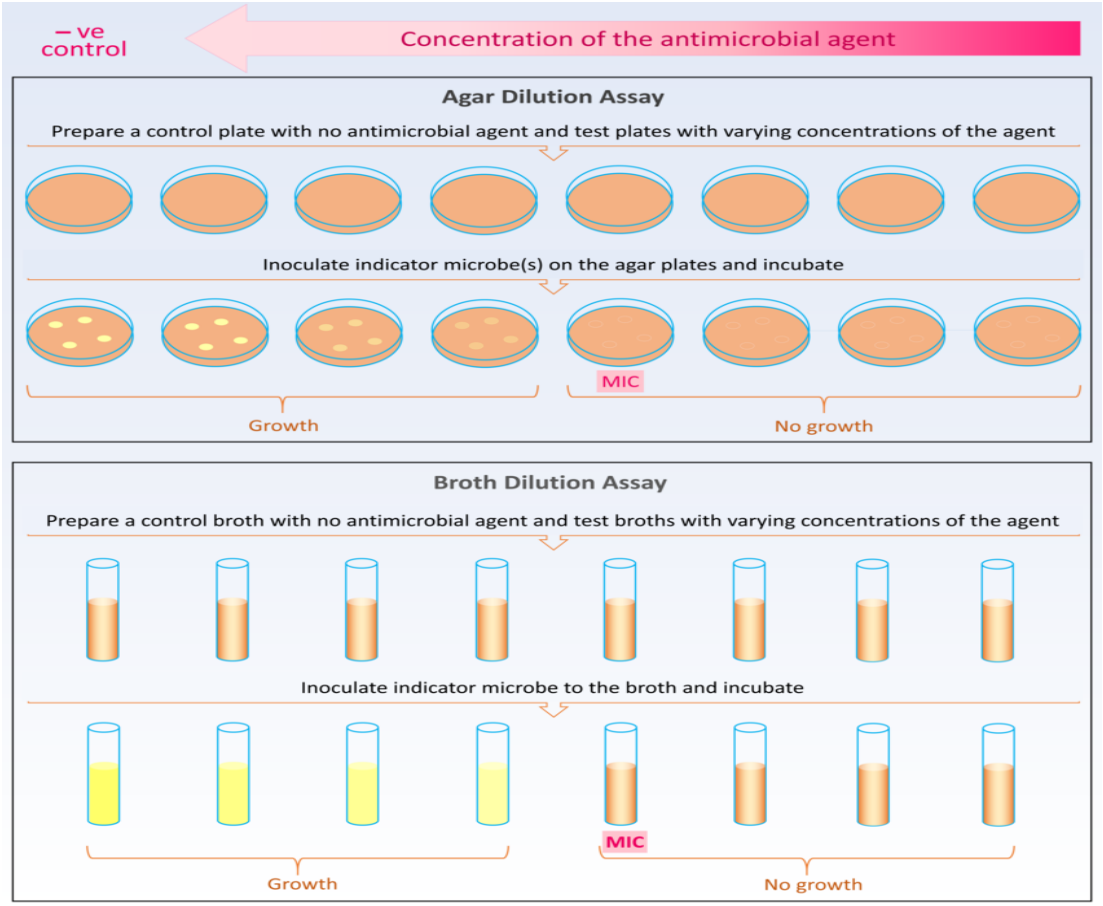

Fig. No. 10: Agar Dilution.

Both the broth dilution and agar dilution techniques are crucial for directing the choice of the best antimicrobial treatment and tracking variations in antimicrobial susceptibility over time. Procedures for broth macrodilution and agar dilution for antibacterial assessment. Agar plates with different antimicrobial agent concentrations are inoculated with the indicator microorganism in an agar dilution procedure. The lowest concentration of the substance that inhibits discernible growth following incubation is known as the minimum inhibitory concentration, or MIC. The indicator microorganism is injected into broth tubes with varying antimicrobial agent concentrations in a process known as broth macrodilution. The lowest concentration at which there is no discernible growth is known as the MIC.

ANTIOXIDANT ACTIVITY

1. FOLIN-CIOCALTEU METHOD

The Folin–Ciocalteu test is a well-known method aimed at determining the total phenolic content (TPC). The Folin-Ciocalteu test was widely used in clinical and nutritional studies to measure the total polyphenolic content in plant-derived foods and biological samples. This method was originally designed to analyse proteins, but it was later adopted by Singleton, Orthofer and Lamuela-Raventos (1999) in order to analyse the phenolic components in wine, after which it became a routine test for the antioxidant evaluation of food and plant extracts

Since the Folin–Ciocalteu test is currently commercially available from a number of significant commercial companies, it is frequently used to measure the amount of polyphenols in foods and beverages as well as extracts obtained from plants. The Folin–Ciocalteu test, which is part of the pharmacopoeia, was accepted as an official method in Europe in 1990 for determining the total phenol level in wines.

The basis of the Folin–Ciocalteu test is the reduction of the Folin–Ciocalteu reagent with alkaline phenolic substances. Although the Folin–Ciocalteu reagent's precise chemical makeup is unknown, it is thought to comprise a compound of phosphomolybdic and phosphotungstic acid that is reduced to produce a blue chromophore with the highest absorption at 765 nm. The complex's core molybdenum ion is recognised as a reducing site, where the phenolic antioxidant donates an electron to decrease the Mo6+ ion to Mo5+. An α-Keggin structure is present in the anionic derivatives of phosphotungstic and phosphomolybdic acids, while the blue complex exhibits a large wheel structure (Mo154) of the cluster type.

The Folin–Ciocalteu test is therefore a SET-based assay that is connected to the phenolic antioxidants' capacity to reduce. The most widely used reference standard is gallic acid, and the TPC findings are typically reported as gallic acid equivalent. It is necessary to standardise the

provided results because the TPC values are sometimes also expressed as catechins, caffeic acid, chlorogenic acid, or the equivalent of ferrulic acid.

Among the many benefits of the Folin–Ciocalteu test for TPC detection are its robustness, simplicity, and reproducibility. It does, however, have certain disadvantages. First of all, the test is sensitive to temperature, pH, and reaction duration; therefore, precise reaction state selection is required for consistent and trustworthy findings. Second, because non-phenolic reducing substances in the system contribute to the reduction of the Folin–Ciocalteu reagent, TPC overestimation is a significant concern for the Folin–Ciocalteu test. Reducing sugars and certain amino acids are two types of pollutants. Therefore, when compared to the values produced by HPLC methods, the TPC measurements may be one size too high. . As a result, TPC measurement values may be one magnitude larger than those derived using HPLC techniques. Furthermore, the test is conducted in aqueous systems, and it has limited applicability for lipophilic phenols—with the exception of situations in which the solvent solution is altered.

A technique for microtitre 96 plates was developed for food samples and urine samples in order to increase the Folin–Ciocalteu test's durability and decrease its expense and duration.

The Folin-Ciocalteu test, which measures the amount of polyphenols in urine, was used as a biomarker of the polyphenol supply and was linked to improvements in the lipid profile and glucose response, a decrease in DNA oxidation, a decrease in cardiovascular risk factors like high blood pressure, and an increase in nitric oxide, a potentially calming agent. An additional benefit was enhanced cognitive function, which was linked to a greater intake of foods high in polyphenols.

B. Alcalde and colleagues (2019) evaluated the antioxidant potential of polyphenols based on their chemical structures. Folin–Ciocalteu (FC), FRAP, and TEAC were among the tests used to identify the polyphenols with varying numbers and locations of hydroxyl groups. Additionally, voltammetric measurements using screen-printed carbon electrodes were made between -0.2 and 0.9 V (in relation to the Ag/AgCl reference electrode) to Examine these compounds' oxidation behaviour. Weak correlations between tests were found, indicating that each compound's behaviour changed depending on the approach taken.

2. TOTAL ANTIOXIDANT CAPACITY (TAC)

The Total Antioxidant Capacity (TAC) assay is a colorimetric method that quantifies the antioxidant potential of a sample based on its ability to reduce copper ions (Cu^{2+}) to cuprous ions (Cu^{+}), which then react with a chromogenic substrate to produce a color change. This assay is widely used in various fields, including food science, pharmacology, and clinical diagnostics.

Materials Needed

- Sample Extract: Prepared from the material of interest (e.g., plant, food, or biological sample)
- Copper(II) Sulfate ($CuSO_4$): Typically 0.1 M solution. • Chromogenic Substrate: Often a colorimetric reagent like neocuproine or bathocuproine.
- Standard Antioxidant: Commonly used standards include Trolox (a water-soluble tocopherol analogue) or gallic acid. • Solvent: Distilled water or appropriate buffer solution.
- 96-Well Microplate: For high-throughput analysis
- Spectrophotometer: Capable of measuring absorbance at the specific wavelength corresponding to the chromogen's maximum absorbance.

Procedure

1. Preparation of Standards and Samples

o Prepare a series of standard solutions of known antioxidant concentrations using Trolox or gallic acid.

o Dilute the sample extract to an appropriate concentration to fall within the standard curve range.

2. Reaction Setup

In each well of a 96-well microplate, add:
50 µL of the standard or sample solution.
50 µL of 0.1 M $CuSO_4$ solution
50 µL of chromogenic substrate solution.
Mix the contents gently by pipetting up and down.

3. Incubation: o Allow the reaction to proceed at room temperature for 30 minutes to 1 hour, depending on the specific assay protocol.

4. Measurement: After incubation, measure the absorbance at the wavelength corresponding to the maximum absorbance of the chromogenic product (commonly around 490 nm).

5. Calculation

Plot a standard curve of absorbance versus antioxidant concentration.
Determine the antioxidant capacity of the sample by comparing its absorbance to the standard curve.
Express the results as µmol Trolox equivalents per gram of sample (µmol TE/g) or other appropriate units

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