

DISTINCTIVENESS OF RESEARCH ACTIVITIES



**RAMAIAH
UNIVERSITY**
OF APPLIED SCIENCES



OVERVIEW OF RESEARCH ON ARECANUT


Pro Vice Chancellor
M.S. Ramaiah University of Applied Sciences
Bangalore - 560 054.




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1. Introduction

Arecanut, also known as betelnut (botanically *Areca catechu* L.), holds a significant position as a lucrative crop in various regions of India, including the Western Ghats, Eastern Ghats, East, and North Eastern areas. The Areca palm, from which these nuts are harvested, can grow to varying heights, typically influenced by the local environmental conditions. It's not uncommon for these palms to reach heights of up to 30 meters, which can present challenges during the nut-harvesting process. Arecanut plays a vital role in the religious, social, and cultural festivities, as well as in the economic activities of the Indian population. Additionally, Arecanut finds applications in traditional Ayurvedic medicine and veterinary treatments. Chewing Arecanut is a customary practice in the Indian subcontinent and its neighboring regions. Although Arecanut production is concentrated in a few specific states, the final product is widely distributed throughout the country. There are primarily two methods of processing Arecanut: the "chali" method, which involves sun-drying ripe nuts, is mainly practiced in the Dakshina Kannada and parts of Uttara Kannada districts. Chali is primarily used in the production of scented supari and is in high demand in Northern India. In contrast, different types of red boiled nuts are prepared in other regions of the state to meet the diverse market demands. Approximately 20 percent of the total Arecanut production in the country is consumed in its ripe fruit form.

Arecanut is transformed into products like pan-masala, gutka, and scented supari, which are experiencing increased popularity within the country. There are more than 150 varieties of Arecanut traded, each varying in terms of ripeness, processing conditions, and taste characteristics to meet the diverse demands of different market centers in the country. The process of sun-drying unhusked ripe nuts to create "chali supari" necessitates exposure to good sunshine for approximately 45 to 60 days to reduce the moisture content to about 10 percent. The Arecanut industry also generates a valuable by-product: the husk of the nuts. This husk can be repurposed for manufacturing particle boards, paper, and other products. Additionally, "Chogaru," another by-product derived

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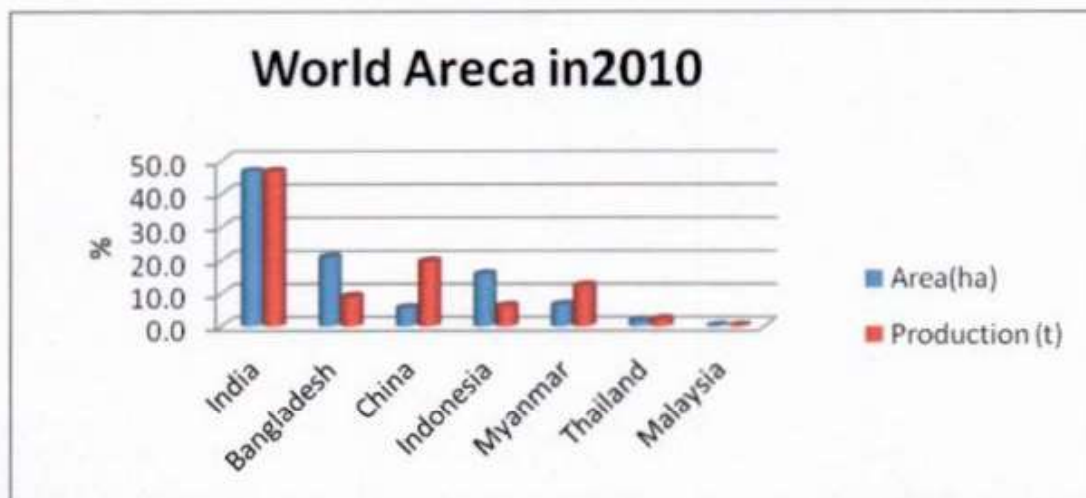




from the processing of tender nuts, is rich in tannins and finds application in the leather industry for the conversion of hides into skins.

2. Global Scenario of Arecanut

In the year 2009-10, global Arecanut production amounted to approximately 1.033 million metric tons, cultivated across an area of 829,000 hectares. India holds the top position in both Arecanut cultivation, covering 47% of the total area, and production, contributing 47% of the global output. Other countries engaged in Arecanut production include Bangladesh, accounting for 21% of the global Arecanut cultivation area and 9% of the total production; China, covering 6% of the cultivation area and contributing 20% of the worldwide production; and Indonesia, with 16% of the global cultivation area and a 6% share in production. Additionally, Myanmar and Thailand also engage in Arecanut cultivation on a smaller scale. The global Arecanut productivity stands at 1.21 metric tons per hectare, and India's productivity aligns with this global figure, reaching 1.27 metric tons per hectare (source: DGCI&S, Kolkata)

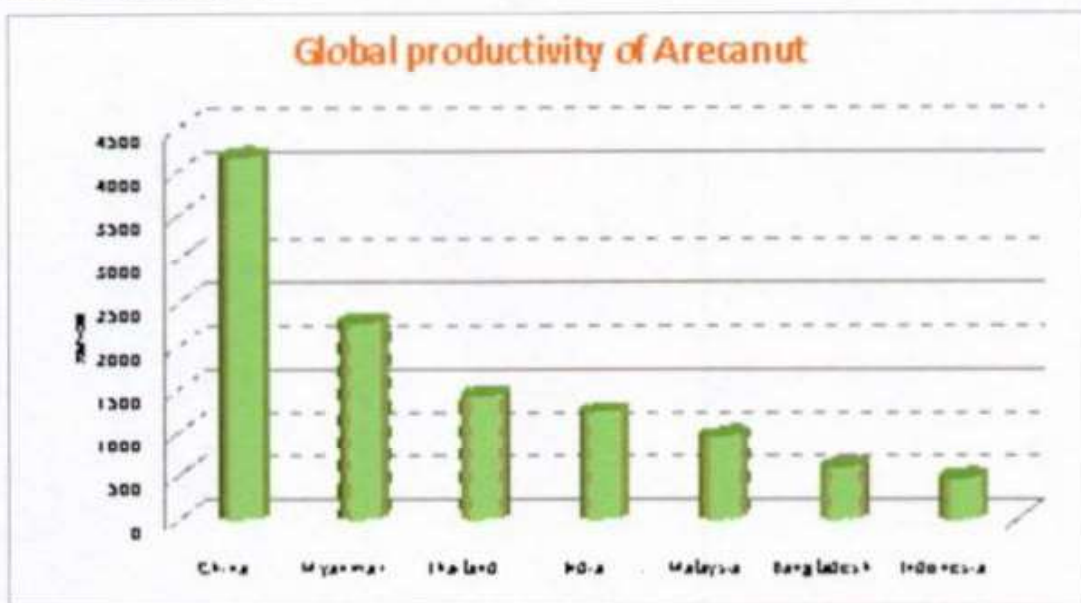


Area and Production Shares of Major Global Producers (Source: Krishisewa)

World Productivity of Arecanut

China leads the way with an impressive productivity of 4,164.76 kilograms per hectare, securing the top spot, while Myanmar claims the second position with a productivity of

2,264.15 kilograms per hectare, closely followed by Thailand. Despite India's prominent position as the largest global Arecanut producer, it ranks fourth in terms of productivity, with Malaysia, Bangladesh, and Indonesia occupying the fifth, sixth, and seventh positions, respectively.



(Source: Krishisewa)

3. Indian Scenario

India holds the dominant position as the primary global producer and consumer of Arecanut. Arecanut production is chiefly concentrated in six states: Karnataka, Kerala, Assam, Meghalaya, Tamil Nadu, and West Bengal. Arecanut thrives as a significant plantation crop in the coastal and southern districts of India, particularly in regions with assured irrigation facilities. The continuously growing demand for Arecanut-derived products such as paan, supari, and gutkha has resulted in a consistent rise in Arecanut prices on a global scale. The methods of processing, the level of ripeness, and consumer preferences play a significant role in determining the types of Arecanut products that are manufactured. In terms of both area and production, Karnataka takes the lead with 46% of the area and 47% of the production, closely followed by Kerala with 24% and 23%.

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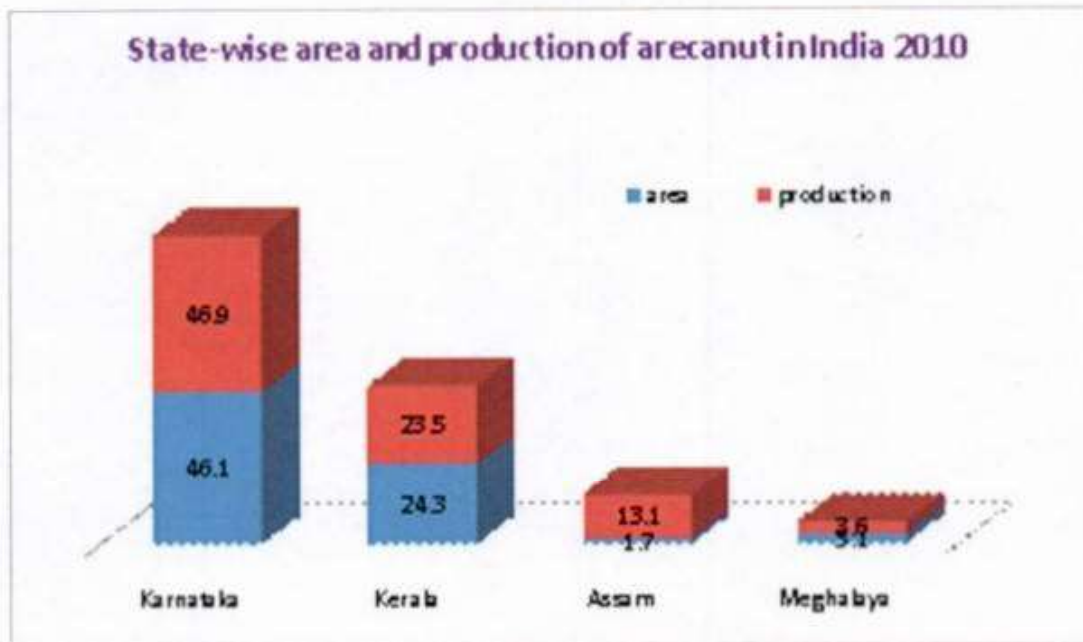
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respectively. On the other hand, Assam, West Bengal, and Meghalaya contribute relatively less in terms of both cultivation area and production shares as of 2010.



Area and Production Shares of Major Arecanut Producing States in India (Source: Krishisewa)

Arecanut is predominantly grown in the states of Karnataka, Kerala, and Assam. Notably, Karnataka and Kerala together contribute to approximately 70 percent of the total Arecanut production in the country. Additionally, other significant Arecanut-producing states, including Tamil Nadu, Maharashtra, Andhra Pradesh, West Bengal, and Odisha, have witnessed a growth in Arecanut cultivation in recent years. Regarding the proportion of cultivation area, Karnataka has experienced an increase from 30 percent to 50 percent between the periods ending in 1980 and 2010. In the same timeframe, Kerala and Assam have seen minor reductions in their relative shares. It's essential to highlight that the actual cultivation area has expanded in all these states.

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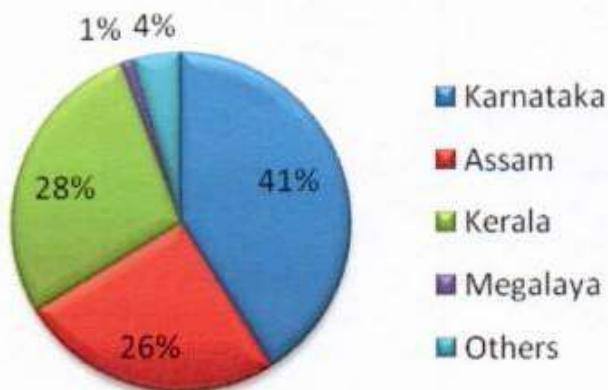
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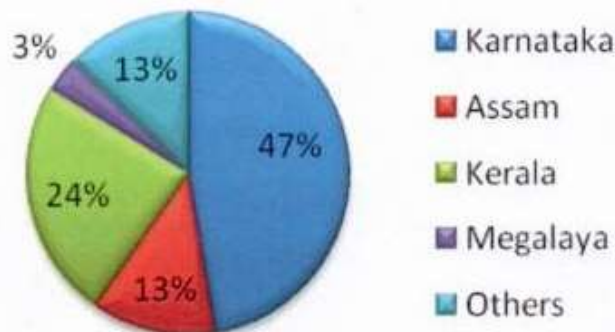
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Triennium ending 1980-81



Triennium ending 2009-10



Source: Indiatat.com

Shifts in Relative Shares of Arecanut Production

Regarding production, Karnataka maintained its top position during the triennium periods of 1980 and 2010, contributing more than 40 percent to the country's total production. Over these two periods, the state's relative production share increased by six percent. On

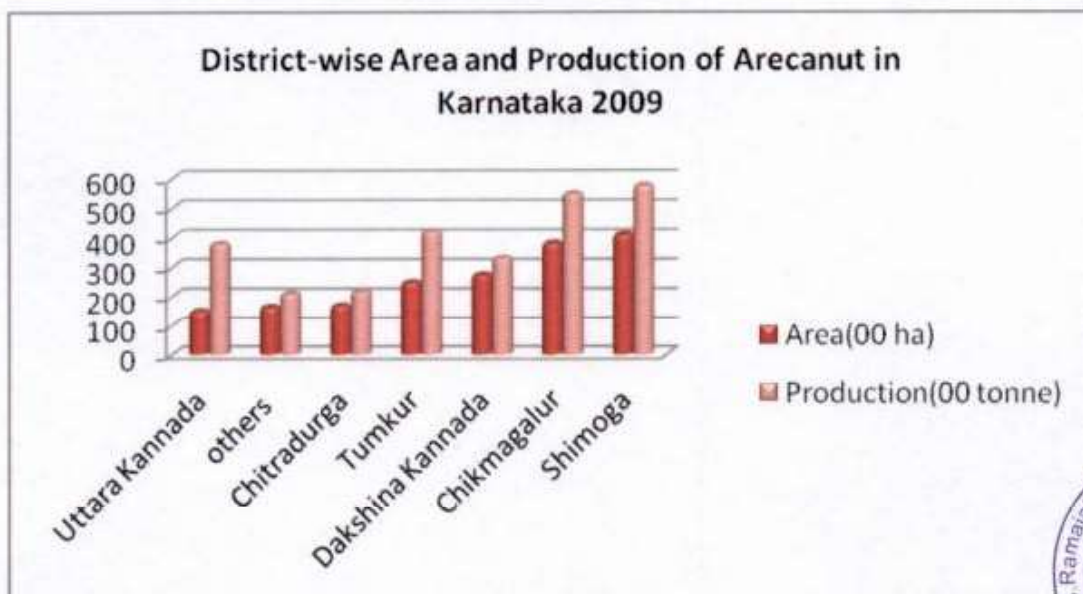
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the other hand, Meghalaya's relative production share increased by two percent, while both Kerala and Assam experienced a decline in their relative production shares.

4. Karnataka Scenario

Karnataka leads the way as the primary Arecanut producer in India, with an expansive cultivation area of about 180.7 thousand hectares. In the 2009-10 period, its production amounted to 269.2 thousand tonnes, making up roughly 45.8 percent of the overall cultivation area and a significant 51.3 percent of the total Arecanut production in the country. It's noteworthy that the Arecanut cultivation area in Karnataka has almost doubled over the course of the last 15 years.



Source: DES, Karnataka 2009

Area and Production Shares of Major Producing Districts in Karnataka

The districts of Shimoga, Chikmagalur, Tumkur Uttara Kannada and Dakshina Kannada are the major arecanut producers of Karnataka which together account for 60 per cent of the area and 65 per cent of production in the state. Shimoga ranks first in area and production (23% and 21%, respectively) followed by Chikmagalur (21% and 20% respectively),

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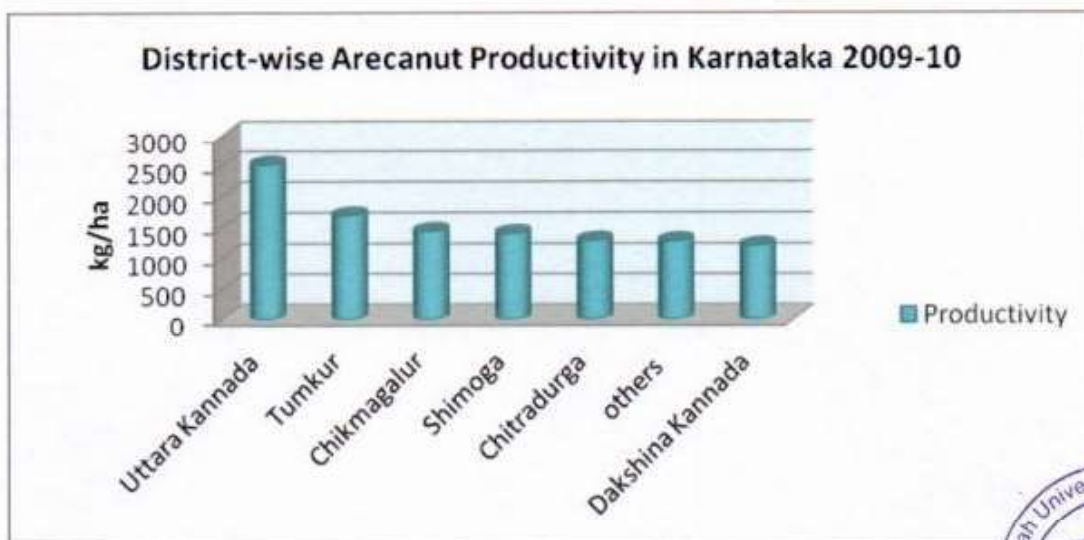
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Dakshina Kannada (15% and 12%, respectively) and Uttara Kannada (8% and 14%, respectively),

District-wise Arecanut Productivity in Karnataka State

Among the major arecanut producers, Uttara Kannada stands first with a productivity of 2516 kg/ha, followed by Tumkur occupying second position with 1692 kg/ha, Chikmagalur is in third position with 1436 kg/ha, on the other hand Dakshina Kannada stands seventh with 1203 kg/ha.



District-wise Arecanut Productivity in Karnataka (Source: Krishisewa)

5. Agronomy

Soil: Arecanut is primarily a plantation crop that thrives in clay loamy soils under the influence of irrigation from tanks. The soil's pH should ideally be slightly acidic to neutral. In cases where the soil pH drops below 5.0, the addition of lime is recommended.

Altitude: The altitudinal range suitable for Arecanut cultivation is influenced to some extent by the geographical location. In the northeastern regions, such as Assam and West Bengal, Arecanut is cultivated on the plains because higher elevations are prone to

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adverse effects from lower winter temperatures. While Arecanut palm can be grown at altitudes of up to 1000 meters above sea level, the quality of the nuts tends to diminish at higher elevations.

Agro-climate: Arecanut thrives in regions with varying rainfall patterns, such as the high rainfall areas like the Malnad region of Karnataka (exceeding 4500 mm) and low rainfall areas like the plains of Karnataka (around 750 mm). In places where there are prolonged dry periods, supplementary irrigation is necessary to support the palm's growth.

Temperature: Arecanut exhibits adaptability to a broad temperature range, with the ability to withstand temperatures as low as 4°C (e.g., Mohitnagar, West Bengal) and as high as 40°C (e.g., Vittal in Karnataka and Kannara in Kerala). However, the palm thrives most in temperature ranges between 25 to 35°C. Severe damage to the foliage can occur in cases of diurnal temperature fluctuations exceeding 5°C, especially in conjunction with low humidity levels.

7. SWOT Analysis

Arecanut, as a perennial plantation crop that involves substantial investment and has a lengthy growth period, encounters numerous challenges across its production and marketing phases. Identifying the key strengths and weaknesses within the industry, along with recognizing opportunities for increased demand and supply, is essential for pinpointing critical areas where improvements are needed. (Source: Krishisewa)

SWOT Analysis for Arecanut sector

Strengths	Weaknesses	Opportunities	Threats
Can be stored for long period without quality deterioration	Expanding cultivation to nontraditional areca area may lead to	Expanding exports to new markets	Cheap imports from neighboring Asian countries.

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	new disease and pest problems		
Presence of alkaloids and tannins which have industrial application	Not large scale research initiatives on alternative uses	Developing value added products which have industrial application	High cost of arecanut may discourage its use in value added products
Large and growing young consumer base	Non availability of skilled laboures and slow mechanization	Developing food/ nutritional supplements	Health hazards of areca based products consumption; ban imposed on Gutkha
Can be grown in different agro ecological regions	Shifting area from food-grains production to arecanut	Intensive cultivation and adoption of resource saving technologies	Traditional pan chewing habit is slowly disappearing in the society

8. Broad scope for study on Arecanut

- 1. Comprehensive Phytochemical Analysis:** To conduct an exhaustive phytochemical analysis of Arecanut to identify and quantify the presence of various chemical compounds, including alkaloids, flavonoids, tannins, and others.
- 2. Determine Carcinogenic Potential:** To investigate the potential carcinogenic effects of Arecanut consumption on human health through a rigorous and scientifically sound research methodology.

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3. **Address Methodological Shortcomings:** To rectify the methodological inadequacies observed in previous research by employing robust and well-designed sampling, experimental, and analytical methodologies.
4. **Scope Enhancement:** To expand the scope of research on Arecanut consumption and its impact on human health, covering a wider range of factors such as regional variations, preparation methods, and genetic predispositions.
5. **Scientific Validation:** To ensure that the research conducted is scientifically rigorous, unbiased, and free from preconceived notions, thereby providing conclusive and reliable results.
6. **Government and Judiciary Reliability:** To deliver research findings that can be relied upon by government agencies and the judiciary for informed decision-making regarding the regulation and litigation related to Arecanut consumption and its potential health risks.
7. **Time-Bound Research:** To execute the research within a predefined timeframe to promptly provide credible data that can be utilized for policymaking and legal proceedings.
8. **Public Health Impact:** To contribute to the understanding of the health implications of Arecanut consumption, leading to evidence-based public health strategies and awareness programs.
9. **Holistic Assessment:** To conduct a holistic assessment of Arecanut's effects, encompassing not only carcinogenic potential but also other health-related aspects, including cardiovascular, neurological, and oral health.
10. **Long-term Implications:** To explore the long-term health implications of Arecanut consumption, including the association with chronic diseases and overall quality of life.
11. **Scientific and Ethical Standards:** To uphold the highest scientific and ethical standards throughout the research process, ensuring the integrity and credibility of the study.

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- 12. Regular Updates for the Judiciary:** To provide periodic research updates to the judiciary to assist in their decision-making for ongoing and future litigations related to Arecanut consumption.
- 13. Knowledge Dissemination:** To share the research findings with the public, health professionals, and policymakers through publications, seminars, and educational programs.
- 14. Policy Recommendations:** To formulate evidence-based policy recommendations aimed at promoting public health and minimizing potential health risks associated with Arecanut consumption.

9. Alternative uses of Arecanut

Many studies have been conducted to find out the alternate use of the crop. The main constituents of arecanut are polyphenols, fat polysaccharides, fibre and protein. Besides these, nuts contain alkaloids viz. arecoline (0.1 - 0.7%) and others in trace amounts such as arecadine, guvacoline and guvacine.

- It was found that tannins, a by-product from the processing of immature nuts find use in dyeing clothes, tanning leather, as a food colour, as mordant in producing variety of shades with metallic salts etc.
- The nuts contain 8-12% of fat, which can be extracted and used for confectionery purposes. The refined fat is harder than cocoa butter and can be used for blending.
- The medicinal properties were described by Vagbhata (in 4th Century AD) as effective against leucoderma, leprosy, cough, fits, worms anemia, obesity.
- Recent studies have shown that arecanut has pharmacological uses viz. Hypoglycemic effect, mitotic activity, antihelminthic activity, cholinomimetic activity etc. However, several studies have also implicated arecanut to cause carcinogenesis.
- Further, arecanut also shows medicinal value in the following lines however these values are yet to be exploited for commercial use.

Key note

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- In the metabolic system as a digestive and carminative Anti-diabetic (Research from Hyderabad Medical College)
 - Used against certain skin diseases
 - Used as aphrodisiac
 - Improves eyesight when used as Thamboola seva
 - Helps in relieving asthma
 - CFTRI Mysore has developed a soft drink concentrate called Pan Supari Nectar
 - For Low Blood Pressure (Old Arecanut)
- Arecanut husk finds use in preparations of hard boards, paperboards, cushions and non-woven fabrics besides being a good source of furfural. But all these are not commercially exploited due to the cost factors.
 - The arecanut leaf sheath could be used for preparation of throwaway cups, plates, plyboards, tea chest, packing cases and suitcases and these are commercially exploited to some extent.

Tanins

Tannins are obtained as a by-product from the process of preparing immature betel nuts for masticatory purposes. It was found that tannic acid from the nut, when mixed with ferrous sulphate in warm distilled water gave black writing ink of acceptable quality. Immature fallen nuts were used for this purpose. Other uses of tannin are as adhesive in plywood industries and as a textile dye.

Fats

The nuts contain 8-12 per cent fat. Fat from arecanut, can be extracted by solvent extraction using hexane. Areca fat has comparable characteristics with hydrogenated coconut oil. Areca fat can be made edible by refining with an alkali. The fat could be softened by fractional crystallization using hexane (25°C) and randomization using

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sodium methoxide, which gave products desirable for use as confectionery fat. Simple blending of areca fat with butter fat and cocoa fat at 3:1 ratio followed by interesterification of areca fat and cocoa fat at 1:1 ratio gave good products acceptable in confectioneries.

Arecanut husk

It is the outer cover of areca fruit. It constitutes 60-80 per cent of the total volume and weight of the fruits (fresh weight basis). It is now being largely wasted except for being used as an inferior fuel and mulch. Several processes have been developed for utilization of areca husk for making hard boards, plastic and brown wrapping paper. Areca husk is used as a substrate for mushroom cultivation. Arecanut husk fibre was generally longer than woolenised jute, goat hair or coir fibre. About 50 per cent of arecanut husk fibre was finer than other fibres and the remaining 50 per cent of fibre was coarser than those fibres. The tenacity value of arecanut husk fibre was comparable with that of goat hair and woollenised jute. Wet weight of arecanut husk fibre was comparable with that of other fibres. The weight and thickness of all fibre reinforced plastic sheets were comparable. The proportion of fibre in the fibre reinforced plastic sheets varied between 7.6 and 9.9 per cent. The proportion of arecanut husk fibre was higher (9.12 per cent) in comparison with that of glass fibre (7.9 per cent), though the thickness and water swelling ie, increase in weight of the sheets by immersion in water for 20 days, values were same.

Areca leaf sheath

Leaf sheath is yet another raw material obtained from the arecanut palm. In a year palm sheds 5-6 leaves. A process has been developed for making plyboards from areca leaf sheath. These boards can be used for making suitcases, fileboards, and tea chests. Leaf sheath cup making machine is available in the market for making arecanut leaf sheath cups of different sizes and shape. Arecanut leaf sheath was found suitable for making plyboards. Two plies of processed arecanut leaf sheaths in combination with an ordinary wood veneer as core glued with urea formaldehyde resin are used for making the



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plyboards. Leaf sheaths obtained from the farm are highly heterogenous having variations in structure, shape and thickness. The rear end is thicker and the two edges are thinner. The thickness at the center ranges from 3.0 to 8.5 mm (average 5.0 mm). A comparatively homogenous piece of fairly uniform thickness and size 50-65 x 20-25 cm can be obtained if a piece of about 10 cm length from either sides along the grain direction, 5 cm from the distal and 10-15 cm from the end across the grain direction are trimmed out from the sheath. Further, to get a flat sheath of uniform thickness and to remove the buck lings of folds, the sheath is flattened under pressure and heat. For this, the sheaths are soaked in water to about 75 per cent moisture and then pressed for 30 min in a hot Plate press at 4 kg/cm² pressure and 110°C temperature. This process gives flat sheaths of 1.0-1.5 mm thickness with about 12 per cent moisture. To prevent fungal growth on the sheath surface, it can be soaked in 1 per cent copper sulphate solution for 24 hr before pressing. The pressed sheaths are then air dried for one hour or longer. The arecanut leaf sheath plyboards made with two veneers of areca sheaths as the faces and one veneer of even an ordinary wood species like Mango as core ply and bonded with Urea formaldehyde resin make commercially acceptable boards with average dry and wet glue shear strengths of 50 kg and 12 kg respectively.

Arecanut stem and leaf

Arecanut stem forms a useful building material in the villages and is widely used in arecanut growing area for a variety of construction purposes. The leaves are good source of organic manure. Their approximate composition is N₂ (0.94 per cent); P₂O₅ (0.096 per cent) and K₂O (1.00 per cent).

Source for the study: Directorate of Arecanut and Spices Development, Calicut, Kerala



Ref. No.: RUAS/DSR/2020/127

Date: 13-October-2020

Place: Bangalore

From
Prof. Govind. R. Kadambi
Acting Vice Chancellor

To
The Director
Dept. of Horticulture
Govt. of Karnataka
Lalbagh, Bangalore - 560004

Dear Sir,


Sub: Research Project Proposal on "*Assessment of Phytochemical and Pesticide Analysis for Arecanut – Measurement of Health, Safety and Efficacy*" to Task Force on Arecanut, Govt. of Karnataka

I am enclosing the research project proposal entitled "*Assessment of Phytochemical and Pesticide Analysis for Arecanut – Measurement of Health, Safety and Efficacy*" for your kind consideration. The Contact details for the above mentioned project will be

1. Prof. V. Madhavan
Mobile No.: 9448087328
Email ID: madhavan.pc.ph@msruas.ac.in
2. Prof. R. Deveswaran
Mobile No.: 9880238650
Email ID: deveswaran.ps.ph@msruas.ac.in

We hope to receive the sanction letter at the earliest.


With Warm Regards



Prof. Govind R. Kadambi

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Research Project Proposal on
"Assessment of Phytochemical and Pesticide Components
for Arecanut – Measurement of Health, Safety & Efficacy"



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Research Project Proposal
on
“Assessment of Phytochemical and Pesticide Components
for Arecanut – Measurement of Health, Safety & Efficacy”
to
Task Force on Arecanut, Govt. of Karnataka



By,
Prof. R. Deveswaran
Associate Professor
Faculty of Pharmacy
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Research Project Proposal on

**Assessment of Phytochemical and Pesticide Components for
Arecanut – Measurement of Health, Safety & Efficacy**

Submitted to

Task Force on Arecanut, Govt. of Karnataka

By



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PROJECT PROPOSAL ON ARECANUT

A Meeting was conducted on 24.9.2020 at Vikasa Soudha, Bangalore with members of Task Force on Arecanut. The meeting was chaired by Honorable Chairman for Task Force, Director, Dept. of Horticulture, Vice-chancellor of Agricultural and Horticulture University, members from Horticulture department, RUAS, GKVK, MAMPCO and press. It was decided in the meeting to proceed further on Research pertaining to Arecanut and a detailed project proposal was requested from RUAS.

Prior to this, a Meeting was held at CAMPCO Ltd. Mangaluru on 2/3/2020 at the initiative of the Task Force on Arecanut constituted by the Govt. of Karnataka. Subsequent to that a Meeting was held at Faculty of Pharmacy, RUAS on 14.03.2020.

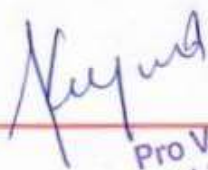
RATIONALE FOR THE STUDY

Research on Arecanut, particularly about the phytochemical analysis and the effects of consumption of Arecanut on human health were inadequate. The available research papers on the effects of consumption of Arecanut were mostly with inadequate methodology and scope. Hence cannot be relied upon by Government and judiciary to come to any definite conclusion that Arecanut consumption has carcinogenic effect. There were shortcomings in sampling, methodologies and design of experiments. The conclusions drawn were not "Scientific" and were influenced by preconceived notions. Therefore, the current project was proposed which would be scientific, holistic, unbiased and time bound. The outcome of this research will also be useful to the judiciary in deriding about litigations from time to time.

RESEARCH PROJECT PROPOSAL ON ARECANUT

The following are the activities are planned:

1. Qualitative studies & chemical analysis on samples collected from 3 Agroclimatic regions and 5 types of samples based on growth stage & treatment process
2. Gas Chromatography and Mass Spectrum analysis (GCMS) of the 15 samples for identification & estimation.



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3. High Performance Thin Layer Chromatography studies (HPTLC) for identification comparing with that of marker compounds
4. In-vitro cancer activity studies on mouth/buccal cavity and stomach using selected samples of Arecanut extracts
5. In-vitro cancer activity studies on mouth/buccal cavity and stomach using standard "Tambula"
6. Pesticide content analysis on Arecanut samples
7. Preparation of formulations of Arecanut as per the "classical Nighantus of Ayurveda"
8. Standardization of "Tambula" (4 varieties of betel leaf) for human consumption
9. Manufacture of products from arecanut namely Mouth refresheners, Gel for wound healing and Anti-ulcer product
10. Organizing a 2 day scientific conference inviting the scientists & scholars from the field of Indian Systems of Medicine, Science & Pharmacy to present and discuss findings relating to Arecanut.

Detailed Proposal on Arecanut Research

The following activities will be co-ordinated by Drug Design and Development Centre, Faculty of Pharmacy, M.S.Ramaiah University of Applied Sciences, Bangalore.

1. Qualitative studies & chemical analysis for various species of Arecanut samples -
This study will be carried out to identify the presence of phytochemical compounds present in different stages of arecanut.

The following phytoconstituents will be tested

- a. Alkaloids
- b. Carbohydrate
- c. Glycosides
- d. Phytosterols


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- e. Tannis and Saponins
- f. Fixed oils and fats
- g. Gums and Mucilage
- h. Volatile oil
- i. Flavanoids
- j. Alcohol and water soluble extractive values
- k. Moisture content by Loss on drying.

2. GC-MS analysis of arecanut samples – The GC-MS analysis will be carried out using GC: Agilent 7890A MS: 5975C MSD, Gas Chromatograph using electron impact ionization, Gas Chromatograph coupled to quadrapole mass analyser, with DB 5 MS of dimensions 30m L x 0.25mm ID x 0.25um film thickness. The samples will be injected and mass spectral scan range was set at 30m/z to 700m/z. The chemical constituents will be identified by GCMS. The fragmentation patterns of mass spectra will be compared with those stored in the spectrometer database using National Institute of Standards (NIST) using Data Analysis software.
3. HPTLC analysis of arecanut samples – To screen and identify the Phytochemical compounds present in different stages of arecanut. High performance thin layer chromatography (HPTLC) studies will be carried out using a Camag HPTLC system with a Linomat V sample applicator, a Camag 3 TLC Scanner and WINCATS 4 software for interpretation of the data. Aluminum plate (20 x 10 cm) precoated with silica gel will be used as adsorbent. The mobile phase will be developed and the sample will run in the mobile phase for a suitable time period. The plates will be then developed in a Camag twin trough chamber and scanned at 254, 366 and 425 nm. The R_f values of the extracts were determined using WINCATS 4 software. The developed plates were photo documented at 254 nm, 366 nm and 425 nm using a Camag 3 Reprostar.


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4. To study the safety of arecanut using cell lines. This will be carried out by carrying out analysis such as
 - a. MTT Assay
 - b. To study the effect of arecanut extract in Liver cancer cell line - HepG2
 - c. Lung cancer cell line - A549
 - d. Colon cancer cell line- Caco-2.
5. Pesticide content analysis of arecanut samples – The presence of pesticide content in the different stages of arecanut will be established by Chromatographic method of analysis such as Gas chromatography. This evaluation will be carried out for Carbosulfan, Chloropyriphos, Cypermethrin, Dichlorovos and Lambda Cyhalothrin etc.
6. Preparation of formulations of Arecanut as per the "Classical Nighantus of Ayurveda"
7. Standardization of "Tambula" (4 varieties of betel leaf) for human consumption
8. Manufacture of products from arecanut namely
 - a. Mouth refresheners
 - b. Gel for wound healing
 - c. Anti-ulcer product.

This work includes product development, manufacture and quality control evaluation of developed products.

9. Organizing a 2 day scientific conference inviting the scientists & scholars from the field of Indian Systems of Medicine, Science & Pharmacy to present and discuss findings relating to Arecanut. This will be planned in the month of June/July 2021


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Duration of Project: 18 months

**BUDGET REQUIREMENTS FOR THE ARECANUT
RESEARCH PROJECT**

S. No	Particulars	Amount in Rs.
1	Qualitative studies & chemical analysis on samples collected from 3 Agroclimatic regions and 5 types of samples based on growth stage & treatment process	2,00,000
2	Gas Chromatography and Mass Spectrum analysis (GCMS) of the 15 samples for identification & estimation.	1,00,000
3	High Performance Thin Layer Chromatography studies (HPTLC) for identification comparing with that of marker compounds.	2,00,000
4	In-vitro cancer activity studies on mouth/buccal cavity and stomach using selected samples of Arecanut extracts	4,00,000
5	In-vitro cancer activity studies on mouth/buccal cavity and stomach using standard "Tambula"	4,00,000
6	Pesticide content analysis on Arecanut samples	1,00,000
7	Preparation of formulations of Arecanut as per the "classical nighantus of Ayurveda"	50,000
8	Standardization of "Tambula" (4 varieties of betel leaf) for human consumption	1,00,000
9	Manufacture of products from arecanut namely Mouth refresheners, Gel for wound healing and Anti-ulcer product	2,00,000
10	Organizing a 2 day scientific conference inviting the scientists & scholars from the field of Indian Systems of Medicine, Science & Pharmacy to present and discuss findings relating to Arecanut.	5,00,000
11	Cost relating to man power to carryout & coordinate the proposed research activities – One Research fellow with Ph.D qualification @ Rs.35,000/- per month for 18 months	6,30,000
12	Miscellaneous documentation & Institutional charges	3,00,000
	Total	Rs.31,80,000
	Rs. Thirty One Lakhs and Eighty Thousand only	

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Time line for the project

Months Objectives↓	PHASE 1		PHASE 2		PHASE 2	
	1-3	4-6	7-9	10-12	13-15	16-18
Qualitative studies, chemical analysis						
Gas chromatography & Mass Spectrum analysis, High Performance Thin Layer Chromatography studies						
In-vitro cancer activity studies on mouth/buccal cavity and stomach of Arecanut extracts; Pesticide content analysis on Arecanut samples;						
Standardization of "Tambula"; In-vitro cancer activity studies on mouth/buccal cavity and stomach of standard Tambula;						
Preparation of formulations of Arecanut as per the "classical nighantus of Ayurveda";						
Manufacture of Mouth refresheners, Gel for wound healing and Anti-ulcer product						

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Deliverables:

PHASE 1:

1. Detailed project report on Qualitative studies & chemical analysis for various species of Arecanut samples
2. Detailed profiling of Arecanut samples by Gas Chromatography and Mass Spectrum (GCMS) and High Performance Thin Layer Chromatography (HPTLC) analysis
3. Report on In-vitro cancer activity studies using cell lines on mouth/buccal cavity and stomach using selected samples of Arecanut extracts

PHASE 2:

1. Report on pesticide content analysis on Arecanut samples
2. Standardization of "Tambula"
3. In-vitro cancer activity studies on mouth/buccal cavity and stomach of standard Tambula

PHASE 3:

1. Development report of Arecanut preparations as per the "Classical Nighantus of Ayurveda"
2. Product development report on products from arecanut such as arecanut namely Mouth fresheners, Gel for wound healing and Anti-ulcer product


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Private University Estd. in Karnataka State by Act No. 15 of 2013
University House, New BEL Road, MSR Nagar, Bangalore - 560 054
Website : www.msruas.ac.in | Ph: 080-4536 6666

Number : 7327/20-21

RECEIPT

Date: 03/11/2020

Received From: KARNATAKA RAJYA ADIKE SAHAKARA SANGA - SHIVAMOGGA

Course : Project - Assessment of Phytochemical and ... Reg. No.:

Sl. No.	Particulars	Amount (₹)
	Tuition Fee	
	Registration Fee	
	Prospectus/Appl/Processing Fee	
	Lib fine/Gym/Other Fe	
	Caution Deposit	
	Others	
		1080000.00
	Total	1080000.00

Amount in Words Ten lakh eighty thousand only

Signature

Cash / Cheque / DD No. : NEFT/SBIN120308225997 & NEFT/SBIN120308173418/3.11.20

Duplicate receipt will not be issued incase loss of original

Seal



K. S. Ramesh

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Ref No.: RUAS/DSR/2023/501

Date: 15th September, 2023

From,

Prof. R. Deveswaran,
Professor,
Department of Pharmaceutics,

To,

Prof. Govind R. Kadambi,
Pro-Vice Chancellor,
Ramaiah University of Applied Sciences,
Bengaluru

Dear Sir,

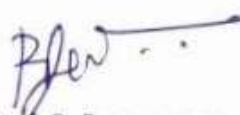
Sub: Research Project on "Assessment of Phytochemical and Pesticide Analysis for Arecanut
– Measurement of Health, safety and Efficacy"

I am happy to inform you about the progress of research work submitted to Task Force on
Arecanut, Govt. of Karnataka. The following activities were carried out-

1. Qualitative studies & chemical analysis for various species of Arecanut samples
2. GC-MS analysis of arecanut samples
3. HPTLC analysis of arecanut samples
4. Other tests related to Arecanut

The abstract of report has been enclosed for your kind information. The detailed report will
be submitted along with final conclusions

With Warm Regards,



Prof. R. Deveswaran



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ABSTRACT OF REPORT

Assessment of Phytochemical and Pesticide Analysis For Arecanut – Measurement Of Health, Safety and Efficacy

Time immemorial, arecanut is chewed in Indian sub-continent due to its medicinal properties. In India, the use of areca nut has been noticed in 1300 BC as cited by Sisu Mayana in 'Anjana Chaitra' and the practice of its chewing from 650 BC as mentioned by Magha in 'Shishupala Vadha'. In India, areca nut and betel leaf are considered as sacred and no ceremonial function is complete without them. Arecanut is chewed in Indian sub-continent and other parts of Southeast Asia due to its medicinal properties. The active principle of arecanut is a pyridine alkaloid, arecoline. Arecoline was reported to have wide pharmacological activities including effects on nervous, cardiovascular, endocrine and digestive system, and anti-parasitic effects, parasympathetic action, vascular-relaxation, urine and electrolyte secretion, ocular effects, antihelminthic activity, prevention of erythrocyte haemolysis, anti-inflammatory/anti-melanogenesis, skin aging and cosmetics, hypolipidemic, platelet aggregation inhibitory activity, prevention of dental cavities, proteasome inhibitors, psychiatric disorders, alzheimer's disease treatment and antimalarial activity. In this work arecanut samples were collected in such a way that the inflorescence part, nuts with different mature stages (ripened, raw and processed arecanut) and leaves from the same plant is collected from 4 different regions of Karnataka namely, Shivamogga, Mangalore Thirthahalli and Chickmagalur. The specimens are trimmed and fixed in herbarium sheets of size 28 x 42 cm. Arecanuts were collected, washed and dried at room temperature.

Aim: The aim of this scientific paper is to assess the phytochemical and pesticide content of Arecanut and evaluate its impact on health, safety, and efficacy.

Objectives:

1. Collect Arecanut samples from four different regions in Karnataka, including the inflorescence part, nuts at various maturity stages, and leaves from the same plant.
2. Perform phytochemical analysis to identify alkaloids, phytosterols, fixed oils, saponins, and phenolic compounds present in Arecanut.



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3. Conduct GC-MS analysis on alcoholic and aqueous extracts of Arecanut to identify 11 specific phytochemical constituents.
4. Determine the percentage of arecoline in Arecanut samples using HPTLC analysis, ranging from 0.12% to 0.95%.
5. Analyze the pesticide content of Arecanut extracts, focusing on Copper, Sulphur, Calcium, Phosphate, and Potassium levels.
6. Conduct in vitro cell line studies using human gingival fibroblasts and human fetal small intestine cell lines to assess the effects of Arecanut extracts on cell viability and the potential mitogenic effects.
7. Prepare and evaluate Arecanut gels with different concentrations of carbopol and xanthan gum for wound healing studies.
8. Perform an acute dermal irritation test following OECD 404 guidelines and carry out wound healing studies using New Zealand White Rabbits and rats to assess the impact of Arecanut gels on wound healing.
9. Develop Arecanut tablets and suspension and evaluate them in vitro.
10. Investigate the potential for healing stomach ulcers using Arecanut extracts through a pyloric ligation model.

Research Findings: The research revealed the presence of various phytochemical constituents in Arecanut, the percentage of arecoline, and the levels of specific pesticides. In cell line studies, it was observed that higher doses of Arecanut extracts had a mitogenic effect on cells, and the extracts exhibited pro-fibrotic potential. Arecanut gels showed promising results in wound healing studies, indicating their potential in promoting wound closure. Additionally, Arecanut extracts demonstrated potential in healing stomach ulcers using a pyloric ligation model.

After complete drying, it was powdered and passed through BSS# 60 and stored in air tight container. Dried powdered drug was used to prepare extract using successive solvent extraction process.

Phytochemical analysis revealed the presence of alkaloids, phytosterols, fixed oils, saponins and phenolic compounds. GC-MS analysis of the arecanut alcoholic and aqueous extracts

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revealed the presence of 11 phytochemical constituents. HPTLC analysis revealed the percentage of arecoline from 0.12 to 0.95%.

The extracts of arecanut were subjected to pesticide content analysis and tested for Copper, Sulphur, Calcium, Phosphate and Potassium. Human Gingival Fibroblast (HGF1-CRL-2014) and human fetal small intestine cell line (FHS 74 Int) were used in the *in vitro* cell line studies of areca nut extracts. Both cell lines were grown as a monolayer culture in complete medium. HGF-1 cells were cultured in T-25 flask in DMEM containing 4 mM L-Glutamine, 4.5 gm/L Glucose, 1mM sodium pyruvate and 1.5 gm/L sodium bicarbonate, 10% FCS, 1% Pen-Strep at 37°C in a 5% CO₂ humidified atmosphere and 95% air in a CO₂ incubator. FHS 74-Int cell lines were cultured in DMEM with high Glucose and EGF.

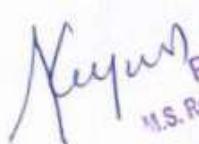
Cells were passaged twice/week under sterile condition. Cell line studies using human gingival fibroblasts revealed that higher doses of the extracts have a mitogenic effect on the cells, evidenced by a plateau patterning at higher doses of the treatment. Most of the cells exhibited maximum cell viability at lower concentrations.

The cell viability percentage varied from 37-80% at the concentration of 2.5mg/ml. The higher doses of the extracts are an indicative of Pro-fibrotic potential of the extracts provided. Gel base was prepared with three different concentrations (0.75, 1, and 1.5%) of carbopol and xanthan gum. 8 formulations were prepared using xanthan gum and carbopol 940 as gelling agent.

The animal study was approved by Institutional animal Ethical Committee (IAEC) of Faculty of Pharmacy (IAEC Ref No.: XXIV/MSRFP/CEU/M-017). New Zealand White Rabbits (either sex) was used for the study. The acute dermal irritation test was performed as per OECD 404 guidelines prior to the wound healing studies using rabbit. Excision and incision wound healing models were carried out using rats for the arecanut gels.

A significant increase in wound healing was observed in arecanut treated groups in comparison to control and standard groups. Records show the reduction of wound area of different groups.

Areca nut tablets and suspension were prepared and evaluated *in vitro*. Pyloric ligation model revealed the healing of stomach ulcers using the arecanut extracts.


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Quantitative Conclusion: This study provides valuable insights into Arecanuts cultivated in different regions of Karnataka and their potential applications. It offers significant information regarding the phytochemical composition, pesticide content, impact on cell viability, wound healing, and stomach ulcer healing. These findings can benefit both the scientific community and farmers seeking to cultivate the right variety of Arecanut.



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