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INVESTIGATION OF THE PHARMACOGNOSY AS WELL AS THE ANTIOXIDANT, ANTI-INFLAMMATORY POTENTIAL OF THE KATHA POWDER

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Keywords:

Acacia catechu, polyphenolics, Katha, DPPH, Nitric oxide

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ABSTRACT: Objective: To investigate the Pharmacognosy as well as the Antioxidant, Anti-inflammatory potential of the Katha powder. **Methods:** The Coarsely dried chips of *Acacia catechu* heartwood were treated with 10% hydro-alcoholic solution to obtain Katha as the final product. The powdered Katha was standardized *via* pharmacognostic parameters. This Kathapower is showing good solubility in hot water, having astringent in the taste. The powder microscopy of the Katha powder is to be demonstrated fragments of acicular crystals, fibers and bordered pitted vessels. Katha powder antioxidant potential is to be accessed by using the DPPH assay and NO Scavenging assay by using ascorbic acid as a standard drug. Further, the Katha powder is to be subjected for the assessment of its anti-inflammatory potential by use of Heat-induced hemolysis as well as the Hypotonicity-induced hemolysis approach by the use of aspirin or diclofenac sodium as a standard drug. **Results:** Microscopical investigations were showed that Katha showing the presence of fragments of acicular crystals, fibers, and bordered pitted vessels. *In-vitro* study shows that the Katha powder has excellent antioxidant as well as anti-inflammatory potential in a dose-dependent manner in comparison to the result of the heartwood of A. catechu. **Conclusion:** So from this investigation, it is to be suggested that the Katha powder is rich in the phenolic compound, and the experimentation study shows the drug is to possesses a good antioxidant as well as anti-inflammatory property.

INTRODUCTION: *Acacia catechu* heartwood is to be used in the preparation of a potent medicinal product is known as a Katha [1]. Katha is a brown color chocolate fracture hard with characteristics odour and having the astringent in taste. Katha having good hot water solubility and insoluble in cold water.

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Katha is rich in Catechin; its content in the powder varies from 20-25% in Katha powder [2]. This Katha has a strong astringent taste due to the high content of tannin as an active compound. Katha content from the heartwood is to be enhanced by the extraction with the 10% hydro-alcoholic solution instead of using the aqueous solution.

Resultantly the yield value of the Katha increases more than the traditional approach used in the extraction of the Katha. Some research has been reported that the Katha powder is showing various pharmacological actions like antioxidant, anti-bacterial, anti-inflammatory, wound healing astringent action [5]. Katha is to be having more

wound healing activity for diabetic patients whose wound is to be healing very slowly [4]. Moreover, the chemical examination manifests that Katha accommodates some of the phenolic components, especially catechin/ epicatechin, quercetin, taxifolin & that is suggested to possess an antioxidant, anti-inflammatory, astringent & anti-diabetic outcome [5]. So the present study is to designed to access the in vitro antioxidant, anti-inflammatory potential of the Katha powder by used a specific approach for its estimation.

Plant Material: The heartwood of the plant was collected in November 2019 from Solan- district of Himachal Pradesh, India, which further was authenticated by Raw material herbarium and museum, NISCAIR, New Delhi, India. A voucher specimen of the plant was preserved in the herbarium for reference (NISCAIR/RHHD/ Consult/2019/3465-66).

Preparation of Katha: The heartwood of the young mature plant of *A. catechu* was dried at room temperature (25 ± 2 °C) for four consecutive weeks and pulverized [6]. Katha was obtained from the heartwood of *A. catechu* by boiling the chips of heartwood with a 10% hydro-alcoholic solution. Trees of elevated girth having white lines on them are favoured. Afresh felled trees further accord higher yields subsequently dried ones. Dead/ Inferior trees are not utilized for extraction operation. This finally concentrated material receives crystallized, over the cooling process [7].

Concentrated material was kept for two days in the refrigerator at the temperature of 8 °C. The crystallized material was then filtered below the negative pressure to withdraw the last fragment of mother liquor sticking to the residue. The residue was cleaned with 15 ml of ice-cold water & it was dried under vacuum to a constant weight. The rectangular shape chips are cut into a biscuit-like shape, termed as Katha. Katha is dried inside the drying chamber for 16-22 days with the cold air. The moisture pills down inside this chamber. It is the final platform of extraction of Katha. This chamber was containing the hot air that accustomed the Katha. Following the ambient drying, it obtained accessible for the packing operation. The steps involved in Katha processing are to be described below in **Fig. 1**.

FIG. 1: DEMONSTRATING THE STEP INVOLVES IN THE PREPARATION OF KATHA

Pharmacognostical Evaluation of Katha:

Organoleptic Evaluation: The organoleptic characters are the various sensory parameters of *A. catechu* (Katha) like shape, size, colour, odour, taste, and fracture of Katha were resolution. It encompasses inferences drench from examination ensued due to impressions on organs of senses [8].

The Percent Yield of Katha after Extraction: To estimate the percent yield of Katha, the heartwood of *A. catechu* (Katha) is to be extracted with 10% of hydro-alcoholic hot distilled water. After achievement of extraction, a concentrated liquid is obtained is kept for two days in a refrigerator at the temperature of 8 °C. In this period, Katha is to be get crystallized. The final crystallized material is to be obtained is to be known as a Katha [9].

The percentage of Katha was determined as:

Weight of chips taken for extraction = X gm

Weight of Katha obtained = Y gm

$$\% \text{ Yield Katha percentage} = Y/X \times 100$$

Histochemical Studies and Powder Microscopy: These inspections were accomplished to realize the inclusions & comprehensive anatomical aspects of the botanical drug (Katha).

Fluorescence Behaviour of the Katha Powder: Fluorescence behaviour concerning botanical drug Katha powder below ordinary light or UV light (UV 366 nm) is to be resolute; consequently, the powder of Katha sample and with different chemical the visibility of changeable colours that are mentioned in the tabulated form in result portion [10].

When physio-chemical boundaries are insufficient as they frequently take place among the Katha as powdered drugs, the botanical material is conceivably recognized since inherent adulterants/ impurities following the fluorescence inspection. Nature of *A. catechu* (Katha) powder among distinct chemical reagents was executed to disclose the phenomenon of phytoconstituents accompanying colour changes below ordinary day-light through special technique [11].

Antioxidant Potential of the Katha Powder:

(a) Antioxidant Activities Assay by DPPH: The DPPH radical scavenging potential assessment elucidated was pursued with slight alterations. The H atom or electron contributing capacity of the Katha powder was estimated against the development of the purple-colored methanol solution of DPPH. This spectro-photometric assay utilized the stable radical, 2, 2-diphenyl-1-picryl hydrazyl (DPPH), as a testing agent. The working solutions of the extracts produced in methanol & distinct concentrations of Katha powder were utilized.

Ascorbic acid was utilized as standard in 0.2-1.0 mg/ml solution. DPPH (0.002%) was processed in methanol & one ml of DPPH solution; 1 ml of sample Katha powder in solution was added.

The solution mixture was supported in the dark for 30 min, and absorbance was estimated at 517 nm. DPPH solution of one ml was utilized as blank [12]. The absorbance was listed & inhibition was deliberated by utilizing the formula designated below.

DPPH scavenging activity (%) = ((Absorbance of the control) - (Absorbance of the sample)) / (The absorbance of the control) × 100

(b) Nitric oxide (NO) Scavenging Technique: NO radical scavenging potential of the specimen can be executed by utilizing Griess [13]. 75 µl of different concentrations of Katha powder is incubated with 75 µl sodium nitroprusside under visible light polychromatic light for 60 min in a 96 - well micro-plate. For control, 75 µl 95% methanol is added in place of samples. 150 µl Griess reagent is then appended to the reaction mixture, and absorbance is recorded spectrophotometrically around 550 nm [14]. Percent NO Scavenging was determined by utilizing the subsequent equation:

NO scavenging activity (%) = ((Absorbance of the control) - (Absorbance of the sample)) / (The absorbance of the control) × 100

Anti-inflammatory potential of the Katha Powder: The blood was possessed from normal human volunteer those which has not confiscated any NSAIDs, which stand (Non-steroidal and Anti-inflammatory medicine) for 2 weeks preceding the investigation & transmitted to the centrifuge tubes. The tubes were separated at very high-speed rotation at 3000 rpm for 10 min & were cleaned three times with equal volume of normal saline. The volume of blood was estimated and regenerate as 10% v/v suspension with normal saline [15].

(a) Heat-induced Haemolysis: Reaction mixture two ml was appraised of one ml-test Kathapowder (100 – 500 µg/ml) & one ml of 10% RBCs suspension; alternatively, the test sample at most saline was attached to the control test tube. Aspirin was utilized as a standard drug for comparison of anti-inflammatory activity. Entire centrifuge tubes accommodated reaction mixture were incubated interior the water bath at 56 ºC for 30 min.

Finally, of the incubation, the tubes were cooled below the water tap. The reaction mixture was separated by rotating at high speed of 2500 rpm for a time of five minutes& the absorbance of the supernatants was confiscate at 560 nm. The investigation was executed three times for the entire specimen [16]. The % inhibition of Haemolysis was deliberated in such a way:

Percentage of inhibition (%) = ((Absorbance of the control) - (Absorbance of the sample)) / (The absorbance of the control) × 100

(b) Hypotonicity-induced Hemolysis: Distinct Kathapowder (100-500 µg/ml), reference & control sample were individually mixed among one ml of PBS, two ml of hypo-saline & 0.5 ml of HRBC suspension. Diclofenac sodium concentration of 100 µg/ml) was utilized as a basic reference medicine. Entire assay mixtures were put interior the incubator at about 37 °C for 30 min of time & rotated at high speed around 3000 rpm. The supernatant liquid was poured off, or the hemoglobin content was predicted by a spectrophotometer at 560 nm.

All the readings were taken three times & percentage hemolysis was predicted by utilizing the formula [17-18].

Percentage of inhibition (%) = ((Absorbance of the control)-(Absorbance of the sample)) / (Absorbance of the control) × 100

RESULTS:

Organoleptic Evaluation: The drug appears in pieces of the wavering proportion of 4 to 4.5 cm in length and 3.5 to 4.5 cm in the breath, yellowish-brown in colour, fracture hard with characteristics odour & astringent in taste as shown in **Fig. 2**.

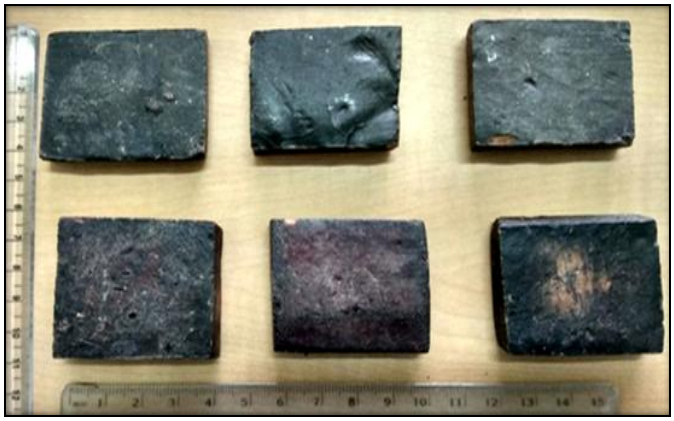


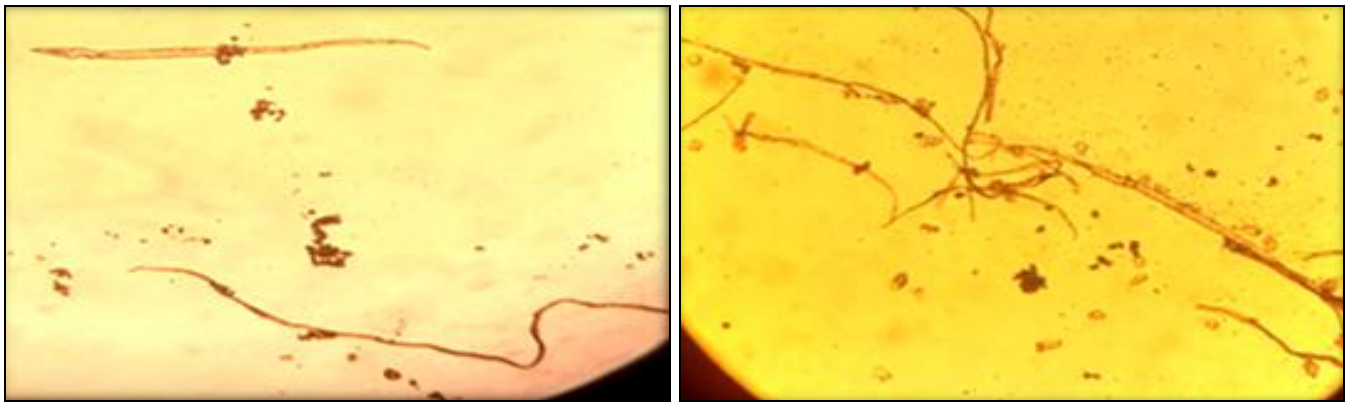
FIG. 2: THE ORGANOLEPTIC CHARACTER OF KATHA

Percent Yield Obtained of Katha after Extraction: The data about Katha content from the heartwood of *A. catechu* is presented in **Table 1**. It is apparent from the table that maximum Katha content of 14.64 percent was acquired and minimum Katha content is 7.95 percent respectively.

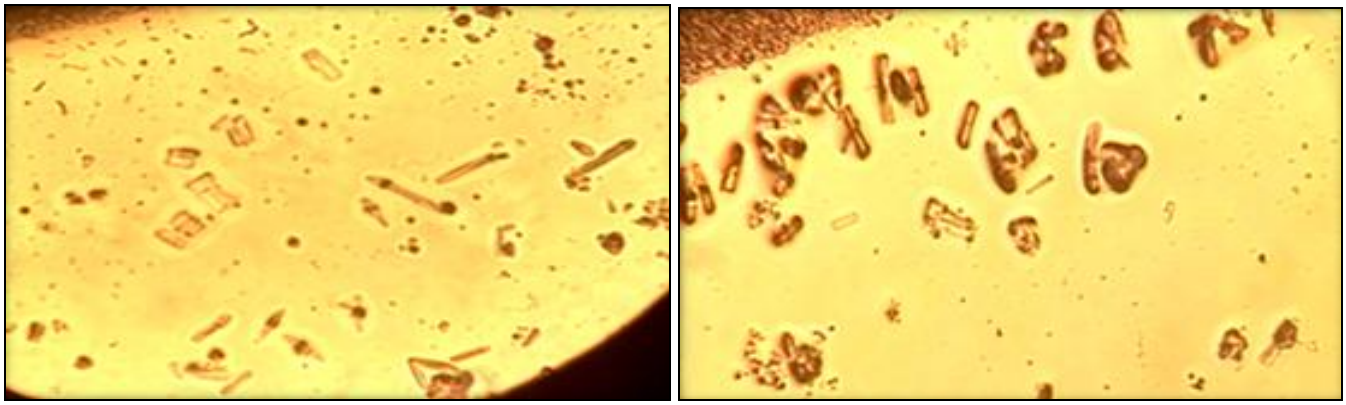
TABLE 1: PERCENT YIELD OBTAINED OF KATHA AFTER EXTRACTION

Trial	Katha extract	% yield w/w	Average value w/w
Trial I	10%hydro-alcoholic hot distilled water	9.12%	11.87%
Trial II	10%hydro-alcoholic hot distilled water	11.95%	
Trial III	10%hydro-alcoholic hot distilled water	14.54%	

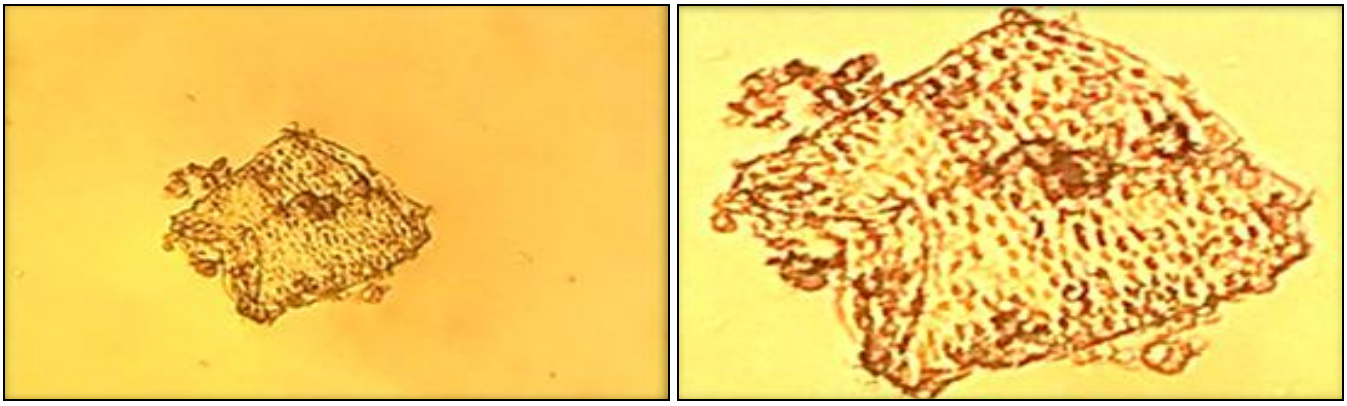
Histochemical Investigations and Powder Microscopy: The powder demonstrated fragments of acicular crystals, fibers, and bordered pitted vessels scattered the powder thoroughly as shown in **Fig. 3**.



FIBERS



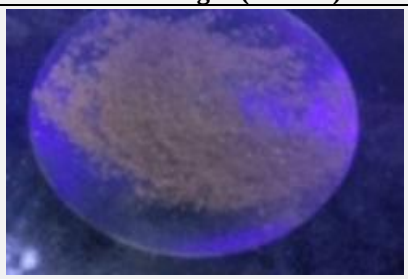
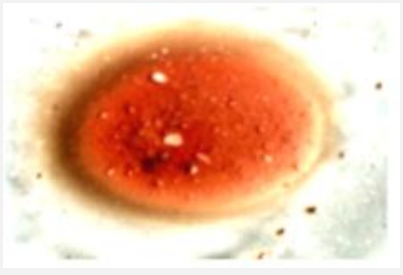
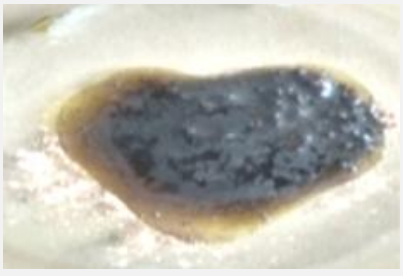
SEVERAL ACICULAR CRYSTALS

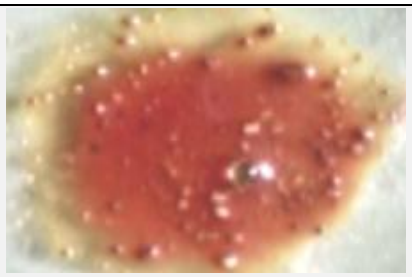
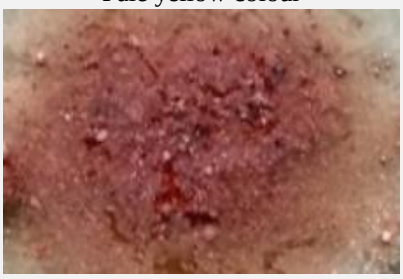
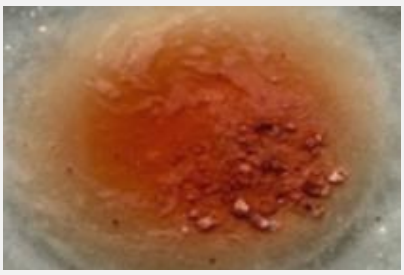
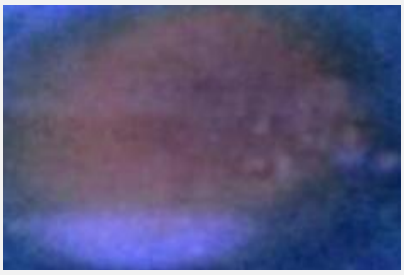
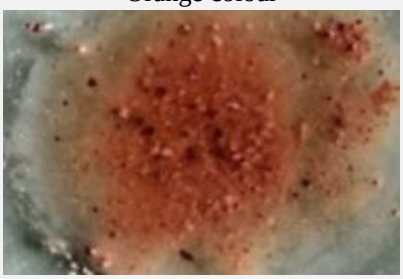
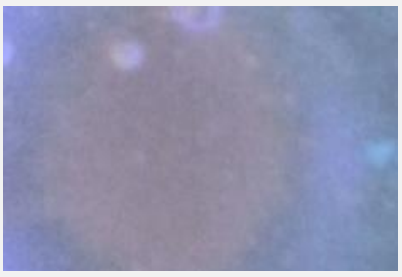


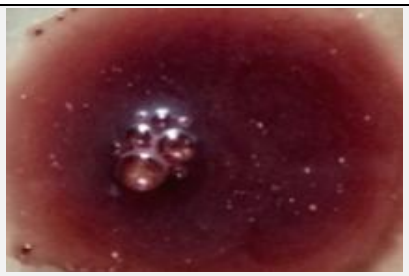
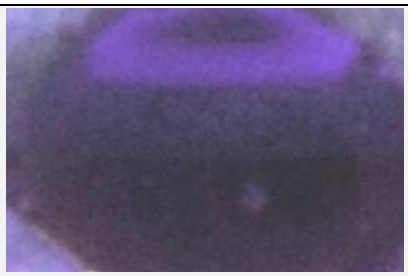
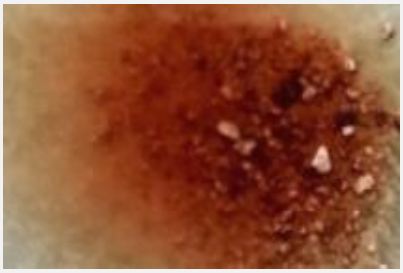
PITTED VESSELS
POWDER MICROSCOPY OF KATHA
FIG. 3: POWDER DEMONSTRATE MICROSCOPY OF KATHA

Fluorescence Examinations of Katha Powder: The examinations are designated underneath; the treatment of powdered drugs with distinct chemical reagents reveals the existence of distinct chemical constituents contemporary in the powdered drugs. The Katha powder is inspected in daylight and UV to detect the fluorescent compounds by the documented technique. A fluorescence examination reveals the existence of chemical constituents with fluorescence character in UV light & colour change inspected in the visible light. The information is described in **Table 2**.

TABLE 2: DATA SHOWING FLUORESCENCE ANALYSIS OF AIR-DRIED DRUG KATHA POWDER WITH DISTINCT CHEMICALS

S. no.	Particular treatment of (Katha)	Under ordinary light	Under UV light (366 nm)
1	Virtually the Powder	 Bark brown in colour	 Bark brown in colour
2	Powder + Acetic acid	 Redish brown colour	 Blackish brown in colour
3	Powder + 5% FeCl3	 Greenish black colour	 Black colour

4	Powder + Iodine	 Pale yellow colour	 Blackish colour
5	Powder + Ammonia	 Redish Brown colour	 Blackish colour
6	Powder + 1N HCL	 Orange colour	 Dark brown colour
7	Powder + H2SO4 (1:1)	 Pale yellow colour	 Dark brown colour
8	Powder + HNO3 (1:1)	 Orange colour	 Dark brown colour
9	Powder + water	 Pale yellow colour	 Blackish colour

10	Powder +1N NaOH (water)	 Redish brown colour	 Black colour
11	Powder + methanol	 Pale yellow colour	 Light brown colour

Antioxidant Potential of the Katha Powder:

Antioxidant Activities Assay (Spectrophotometric Analysis) by DPPH: The more frequent basis utilization of DPPH assay is straightforward and extremely precise. DPPH is depreciated in the radical form through its strength. The present radical appears a secure absorption maximum at a wavelength of 517 nm (purple). In the existence of antioxidants, the color turns from purple to yellow. Consequently, the sole apparatus essential for the assay is a UV- Vis Spectro-photometer. The DPPH free radical scavenging capabilities of Katha powder at distinct concen-trations were estimated and contrasted with that of the standard ascorbic acid **Table 3**. Five distinct working solutions of three Katha powders were utilized, having varying concentrations (0, 250, 500, 750 & 1000 µg/ml). Decolouration due to reaction of antioxidant in samples with the stable DPPH free radical detected by spectrophotometrically. It was perceived that as the concentration of samples enhances, the percentage of free radical scavenging potential also be enhanced. The antioxidant consequence of botanical products is primarily due to the radical scavenging potential of phenolic compounds like flavonoids, polyphenols, tannins, and phenolic compounds. When these compounds enhanced in dose, the antioxidant potential enhanced correspon-dingly in all the samples, as shown in **Table 3**.

TABLE 3: DPPH FREE RADICAL SCAVENGING POTENTIAL IN (%) OF KATHA POWDER & ASCORBIC ACID

Test Sample	Concentration (mg/ml)									
	0		250		500		750		1000	
	Max (517)	%	Max (517)	%	Max (517)	%	Max (517)	%	Max (517)	%
Katha powder	1.654	0%	1.378	16.68%	0.987	40.32%	0.418	74.72%	0.113	93.16%
Ascorbic Acid	1.849	0%	1.341	27.47%	0.452	75.55%	0.079	95.72%	0.072	96.10%

Around the entire Katha powder sample samples investigations, the Katha powder appearing in the concentration of 1.0 mg/ml exhibited the optimum free radical scavenging potential of 93.16%. Correspondingly, in 0.75 mg/ml, Katha powder exhibited the optimum free radical scavenging potential (74.72 %). Katha powder at 0.50 mg/ml had the highest free radical scavenging potential (40.32%). 0.25 mg/ml of Katha powder had optimum free radical scavenging potential (16.6%) It was additionally recognized that the entire tested samples appeared lower DPPH radical scavenging potential when collating with the standards.

The optimum free radical scavenging potential was acquired for the ascorbic acid at 1 mg/ml was raise to be 96.10%.

Additionally, **Fig. 4** is demonstrating that the scavenging percentage of Katha powder was in increasing sequence with the increase in concentration.

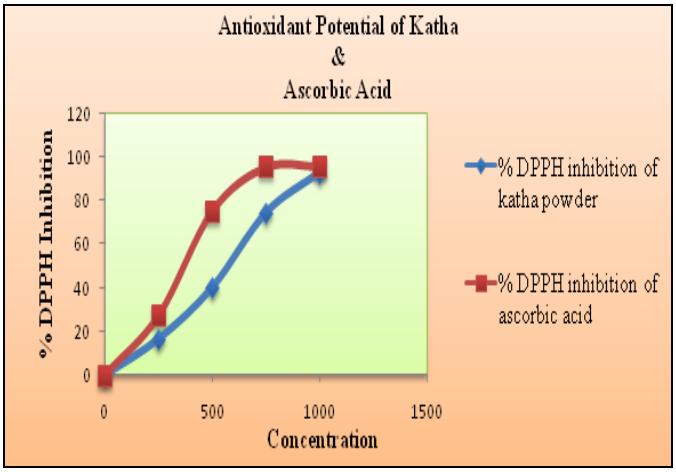


FIG. 4: DEMONSTRATE THAT THE DPPH SCAVENGING PERCENTAGE OF KATHA POWDER & ASCORBIC ACID

Nitric oxide (NO) Scavenging Technique: Nitric oxide radical accused from sodium nitroprusside in aqueous solution at physiological pH connect among the oxygen to generate nitrite ions which were deliberated by Griess reaction.

Nitric oxide radical accused from nitroprusside at physiological pH was accomplished to be inhibited by the distinct extract *e.g.* Katha phytosomes and ascorbic acid as manifested in **Table 4**. Four diverse working solutions of three extracts phytosomes Katha powder and ascorbic acid were utilized having diverse concentrations (0, 250, 500, 750, and 1000 µg/ml) were utilized.

Decolouration due to reaction of antioxidant in samples with the nitric oxide free radical was deliberated by spectrophotometrically. It was perceived that when the concentration of samples enhanced, the percentage nitric oxide scavenging potential also enhanced as shown in **Table 4**.

TABLE 4: PERCENTAGE NITRIC OXIDE SCAVENGING ACTIVITY OF KATHA POWDER & ASCORBIC ACID

Test Sample	Concentration (mg/ml)									
	0		250		500		750		1000	
	Max (546)	%	Max (546)	%	Max (546)	%	Max (546)	%	Max (546)	%
Katha powder	2.654	0%	1.976	25.54%	1.356	48.90%	1.145	56.85%	0.984	62.92%
Ascorbic Acid	2.432	0%	1.842	24.25%	0.830	65.87%	0.095	96.90%	0.042	98.27%

Around the entire Katha powder sample samples investigations, the Katha powder appearing in the concentration of 1.0 mg/ml exhibited the optimum free radical scavenging potential of 62.92%.

Correspondingly, in 0.75 mg/ml Katha powder exhibited the optimum free radical scavenging potential (56.85%).

Katha powder at 0.50 mg/ml had the highest free radical scavenging potential (48.90%). 0.25 mg/ml of Katha powder had optimum free radical scavenging potential (25.54%).

It was additionally recognized that the entire tested samples appeared lower DPPH radical scavenging potential when collating with the standards.

The optimum free radical scavenging potential was acquired for the ascorbic acid at 1 mg/ml was raise to be 98.27%.

Additionally, **Fig. 5** is demonstrating that the NO scavenging percentage of Katha powder was in increasing sequence with the increase in concentration.

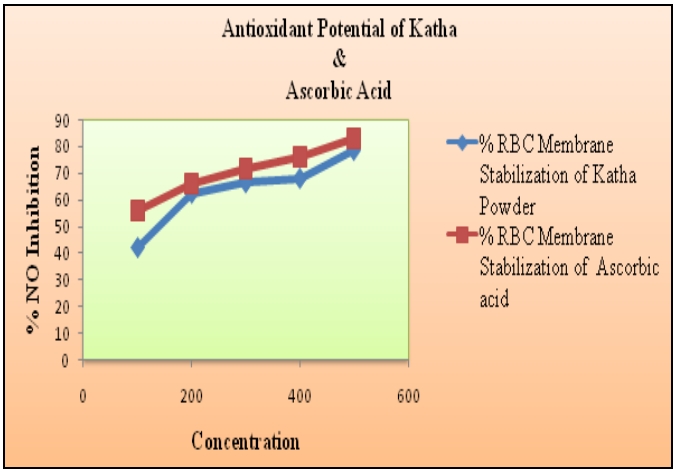


FIG. 5: DEMONSTRATE THAT THE NO SCAVENGING PERCENTAGE OF KATHA POWDER & ASCORBIC ACID

Anti-inflammatory Potential of the Katha Powder: Stabilization of the cell membrane of RBCs when asserting with direct controlled heat was investigated to access membrane stabilization potential of diverse drugs concentration in collation to aspirin. The Katha powder was efficacious in inhibiting heat-induced hemolysis at diverse concentrations. This result is demonstrated in graphical form in **Table 5**.

TABLE 5: EFFECT OF KATHA POWDER ON HEAT-INDUCED HEMOLYSIS OF ERYTHROCYTE

Concentration (µg/ml)	Absorbance at 560 nm	
	Aspirin	Katha powder
500	0.044	0.053
400	0.052	0.086
300	0.065	0.098
200	0.076	0.103
100	0.086	0.124
Concentration(µg/ml)	**% Inhibition of Heat-induced Hemolysis**	
500	85.57	82.62%
400	82.95	71.80%
300	78.68	67.86%
200	75.08	66.22%
100	71.80	59.34%

The control showed absorbance at 560 nm (0.305) used to compare the % Inhibition of Heat-induced Hemolysis

The results demonstrated that Katha powder at concentrations 400 and 500 µg/ml protects the erythrocyte membrane significantly against lysis induced by heat. Katha powder revealed excellent consequence comparable to standard giving percent inhibition of hemolysis value of 82.62% as compared to standard 85.57 % at the concentration of 500 µg/ml. **Table 5**, represents the results obtained for various concentrations of test and standard. The control showed absorbance of (0. 305). Additionally, **Fig. 6**, revealed that the percentage inhibition of heat-induced hemolysis of Katha powder was in increasing sequence with the increase in concentration.

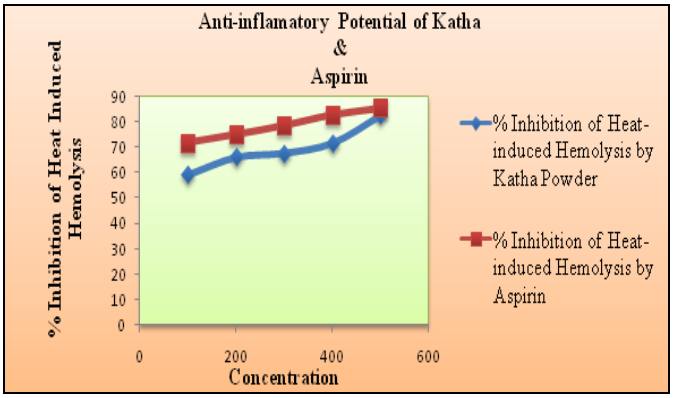


FIG. 6: DEMONSTRATE THE HEAT-INDUCED HEMOLYSIS OF KATHA POWDER WITH ASPIRIN AS A STANDARD

Hypotonicity Induced Haemolysis: The RBC membrane stabilization was repeatedly tested by changing related conditions for hemolysis. The consequences manifested that Katha powder of at concentration range of 200-500 µg/ml shield, represented below in **Table 6**.

Diclofenac sodium (100-500 µg/ml) provided remarkable protection across the damaging ramification of hypotonic solution. Through the concentration of 500 µg/ml, Katha powder manifested a maximum of 69.83, 64.91, 67.54 protection, whereas, Diclofenac sodium (500 µg/ml) revealed 83.27% inhibition of RBC hemolysis when correlating with control. The control showed absorbance of (0.305). Additionally, **Fig. 7** is demonstrating that the % RBC Membrane Stabilization of Katha powder was in increasing sequence with the increase in concentration.

TABLE 6: EFFECT OF KATHA POWDER ON HYPO-TONICITY INDUCED HEMOLYSIS OF ERYTHRO-CYTE

Concentration (µg/ml)	Absorbance at 560 nm	
	Diclofenac sodium	Katha powder
500	0.051	0.065
400	0.072	0.097
300	0.086	0.102
200	0.103	0.113
100	0.152	0.176
Concentration (µg/ml)	**% RBC Membrane Stabilization**	
500	83.27	78.68%
400	76.39	68.19%
300	71.80	66.55%
200	66.22	62.95%
100	56.16	42.29%

The control showed absorbance at 560 nm (0.305) used to compare the % RBC Membrane Stabilization

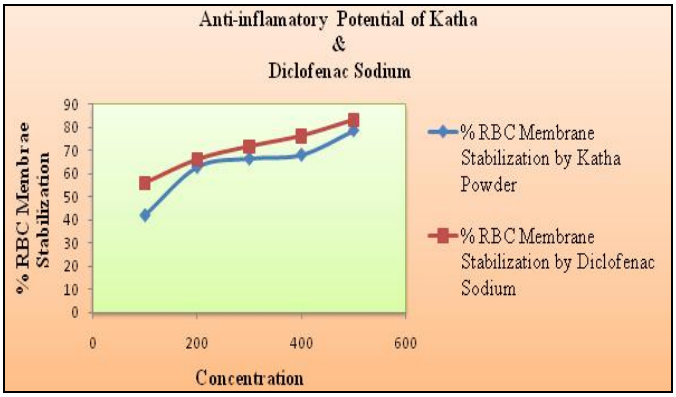


FIG. 7: DEMONSTRATING THE HYPO-TONICITY INDUCED HEMOLYSIS OF, KATHA POWDER WITH DICLOFENAC SODIUM AS A STANDARD

DISCUSSION: Katha obtained by boiling the heartwood of *A. catechu* with a 10% hydro-alcoholic solution increased the percentage yield value up to 12% w/w. Traditionally the aqueous extract is used for the production of the Katha from the heartwood, having yield value 6-7%.

In this study, the new approach can be accelerated the yield value of the Katha. The powder microscopy of the Katha powder is to be demonstrated fragments of acicular crystals, fibers, and bordered pitted vessels it is the good diagnostic character of the Katha. When these compounds enhanced in dose, the antioxidant potential enhanced correspondingly in all the samples. It is to be observed that the Katha powder is to shows comparable antioxidant potential in the comparison of the ascorbic acid. This study is to be suggested that the drug is to possesses excellent antioxidant potential. Further, the Katha *in-vitro* anti-inflammatory study is to be suggested that the Katha powder is to possesses good action against the inflammatory disorder in the body. This study is to be recommended that the Katha powder is to be good antioxidant and anti-inflammatory action to cure various body disorders instead of using the *Acacia Catechu* heartwood.

CONCLUSION: This study is to be presented us that the drug yield value can be enhanced by using the extraction of such type of the modified method. Since the yield value with the traditional method is to be only 5% but using the 10% alcoholic solution, it can be increased upto 10-12%. Katha, as traditional methods of extraction along with the value of the high yield it also has shown the high content of the tannin, flavonoids & phenolic compounds. DPPH and NO radical scavenging approaches show the good antioxidant potential of the Katha powder. Further, the heat-induced, as well as hypo-tonicity induced approach demonstrates that the drug has good anti-inflammatory activity in the human body.

The result of the investigation is to be suggested that the Katha powder is rich in the phenolic compound, and the experimentation study shows the drug is to possesses a good antioxidant as well as anti-inflammatory property.

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