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

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Received: 06 April 2021, Revised and Accepted: 05 May 2021

ABSTRACT

Objective: The objective of the study was 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 through pharmacognostic parameters. This Katha powder is showing the good solubility in the 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 2, 2-diphenyl-1-picryl hydrazyl assay and NO Scavenging assay using ascorbic acid as a standard drug. Further, the Katha powder is to be subjected for the assessment of its anti-inflammatory potential by the use of heat-induced hemolysis as well as hypotonicity-induced hemolysis approach by the use of the 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 of the result of 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 that the drug is to possess a good antioxidant as well as anti-inflammatory property.

Keywords: *Acacia catechu*, Polyphenolics, Katha, 2, 2-diphenyl-1-picryl hydrazyl, Nitric oxide.

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 odor and having the astringent in taste. Katha having good hot water solubility and insoluble in cold water. Katha is rich in Catechin, its content in the powder is varies from 20 to 25% in Katha powder [2]. This Katha having 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% hydroalcoholic 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 such as antioxidant, antibacterial, anti-inflammatory, wound healing, and astringent action [3]. 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 and that is suggested to possess an antioxidant, anti-inflammatory, astringent, and antidiabetic outcome [5]. Hence, the present study is to design to access the *in vitro* antioxidant, anti-inflammatory potential of the Katha powder by used a specific approach for its estimation.

METHODS

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 4 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 favored. 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 2 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 and 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 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.

Pharmacognostical evaluation of Katha
Organoleptic evaluation
The organoleptic characters are the various sensory parameters of *A. catechu* (Katha) such as shape and size; color, odor, 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 hydroalcoholic hot distilled water. After achievement of extraction, a concentrated liquid is obtained is kept for 2 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 g
Weight of Katha obtained = Y g

% Yield Katha = \( \frac{Y}{X} \times 100 \)

Anti-inflammatory potential of the Katha powder

The blood was possessed from normal human volunteer those which has not confiscated any NSAIDs which is stand (nonsteroidal and anti-inflammatory medicine) for 2 weeks preceding the investigation and transmitted to the centrifuge tubes. The tubes were separated at very high-speed rotation at 300 rpm for 10 min and were cleaned 3 times with equal volume of normal saline. The volume of blood was estimated and regenerate as 10% v/v suspension with normal saline [15].

Heat-induced hemolysis

Reaction mixture 2 ml was appraised of 1 ml-test Katha powder (100–500 µg/ml) and 1 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°C for 30 min. Finally, of the incubation, the tubes were cooled below the water tap. The reaction mixture was separated by rotating at a high speed of 2500 rpm for a time of 5 min and the absorbance of the supernatants were confiscate at 560 nm. The investigation was executed 3 times for the entire specimen [16]. The % inhibition of hemolysis was deliberated in such a way:

\[
\text{Percentage of inhibition} (\%) = \left( \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{The absorbance of the control}} \right) \times 100
\]

**RESULTS**

**Organoleptic evaluation**

The drug appears in pieces of the wavering proportion of 4–4.5 cm in length and 3.5–4.5 cm in the breadth, yellowish-brown in color, fracture hard with characteristics odor, and astrigent in taste as shown in Fig. 2.

Percent yield obtained of Katha after extraction

The data about Katha content from the heartwood of *A. catechu* are presented in Table 1. It is apparent from the table that maximum Katha content of 14.64% was acquired and minimum Katha content is 7.95%, respectively.
Histochemical investigations and powder microscopy
The powder demonstrated fragments of acicular crystals, fibers, and bordered pitted vessels scattered thoroughly the powder as shown in Fig. 3.

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 and color change inspected in the visible light. The information is described in Table 2.

Antioxidant potential of the Katha powder
Antioxidant activities assay (spectrophotometric analysis) by DPPH
The more frequent basis utilization of DPPH assay is straight forward 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-visible spectrophotometer. The DPPH free-radical scavenging capabilities of Katha powder at distinct concentrations were estimated and contrast 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, and 1000 µg/ml). Decoloration 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 such as flavonoids, polyphenols, tannins, and phenolic compounds. When these compounds enhanced in dose, the antioxidant potential enhanced correspondingly in all the samples as shown in Table 3.

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

NO scavenging technique
NO 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. NO radical accused from nitroprusside at physiological pH was accomplished to be inhibited by the distinct extract, for example, Katha phytosomes and ascorbic acid.
| S. No. | Particular treatment of (Katha) | Under ordinary light | Under UV light (366 nm) |
|--------|---------------------------------|----------------------|-------------------------|
| 1.     | Virtually the Powder            | Bark brown in color  | Bark brown in color     |
| 2.     | Powder + Acetic acid            | Reddish brown color | Blackish brown in color |
| 3.     | Powder + 5% FeCl3               | Greenish black color | Black color             |
| 4.     | Powder + Iodine                 | Pale yellow color   | Blackish color          |
| 5.     | Powder + Ammonia                | Pale yellow color   | Blackish color          |
| 6.     | Powder + 1N HCL                 | Reddish Brown color | Blackish color          |
| 7.     | Powder + H₂SO₄ (1:1)            | Orange color        | Dark brown color        |
| 8.     | Powder + HNO₃ (1:1)             | Pale yellow color   | Dark brown color        |
| 9.     | Powder + water                  | Orange color        | Dark brown color        |

(Contd...)
Sharma and Raju
Asian J Pharm Clin Res, Vol 14, Issue 6, 2021, 125-132

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. Decoloration due to reaction of antioxidant in samples with the NO free radical was deliberated by spectrophotometrically. It was perceived that when the concentration of samples enhanced, the percentage NO scavenging potential also enhanced as shown in Table 4.

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 and was raise to be 98.27%. In addition, Fig. 5 is demonstrating that the NO scavenging percentage of Katha powder was in increasing sequence with the increase in concentration.

**Table 2: (Continued)**

| S. No. | Particular treatment of (Katha) | Under ordinary light | Under UV light (366 nm) |
|--------|--------------------------------|---------------------|------------------------|
| 10.    | Powder + 1N NaOH (water)       |                     |                        |
|        |                                 | Reddish brown color | Black color            |
| 11.    | Powder + methanol               |                     |                        |
|        |                                 | Pale yellow color   | Light brown color      |

**Table 3: DPPH free radical scavenging potential in (%) of Katha powder and ascorbic acid**

| Test sample  | Concentration (mg/ml) | 0 | 250 | 500 | 750 | 1000 |
|--------------|-----------------------|---|-----|-----|-----|------|
|              | 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 |

**Fig. 4: Demonstrate that the DPPH scavenging percentage of Katha powder and ascorbic acid**

**Antioxidant Potential of Katha and Ascorbic Acid**

- % DPPH inhibition of katha powder
- % DPPH inhibition of ascorbic acid

heat induced hemolysis

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.

The results demonstrated that Katha powder at concentrations 400 and 500 µg/ml protects significantly the erythrocyte membrane 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, represent the results obtained for various concentrations of test and standard. The control showed absorbance (0.305). In addition, Fig. 6 revealed that the percentage inhibition of heat-induced hemolysis of Katha powder was in increasing sequence with the increase in concentration.
Hypotonicity induced hemolysis

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, and 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). In addition, Fig. 7 is demonstrating that the % RBC Membrane Stabilization of Katha powder was in increasing sequence with the increase in concentration.

DISCUSSION

Katha obtained by boiling the heartwood of A. catechu with a 10% hydroalcoholic solution increased the percentage yield value up to 12% w/w. Conventionally, 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 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 show comparable antioxidant potential in the comparison of the ascorbic acid. This study is to be suggested that the Katha powder is to possess excellent antioxidant potential. Further, the Katha in vitro anti-inflammatory study is to be suggested that the Katha powder is to possess 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 the various body disorders instead of using the A. catechu heartwood.

CONCLUSION

This study is to be presented us that the drug yield value can be enhanced 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 up to 10–12%. Katha as traditional methods of extraction along with the high yields value it also has shown the high content of the tannin, flavonoids, and 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 is having a 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 that the drug is to possess a good antioxidant as well as anti-inflammatory property.

ACKNOWLEDGMENTS

The authors extend thanks to Dr. Athar Javed and Mr. Tarapati Rana, Faculty of Pharmacy, Government Pharmacy College Seraj, Mandi (HP) for his technical support to carry out this research study.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial, or otherwise.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

AUTHOR FUNDING

Not applicable.

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