RESEARCH ARTICLE

# Therapeutic and Biological Aspects of Leaf Extracts from Indian Copper leaf Plant (*Acalypha Indica*)

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## Abstract

*Acalypha indica* is found extensively in India and the Indian subcontinent. The whole plant has medicinal values with many ethnobotanical importance which has been described in many ancient and modern literatures. Most medicinal and therapeutic capabilities are present in leaves compared to other plant parts such as roots, stems, seeds and flowers. Various studies have been proposed to establish the therapeutic capabilities of the Indian Copper leaf plant (*Acalypha indica*). This research paper focuses on studying different phytochemicals present in acetone and hydro-alcohol leaf extracts of *Acalypha indica* with quantification of Phenol and Flavonoid content in the plant extracts. This experimental evidence also quantifies the antioxidant properties by DPPH methodology for given extracts and the plants' importance as antibacterial and anti-inflammatory activities. The study also gives insight into the capability of the solvent extracts for the greener synthesis of silver nanoparticles (AgNP's) from molecular silver solution with characterization and morphological characteristics of synthesized silver nanoparticles.

**Keywords:** *Acalypha indica,* Antibacterial, Antioxidant, Anti-inflammatory, Green synthesis, Silver nanoparticles.

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## Introduction

Plants contain many chemical compounds in almost all parts such as leaves, stems, roots, bark, etc. Modern medicinal and herbal technology are derived based on natural and natural derived compounds. Irrespective of many disadvantages such as lower absorption, bioavailability, purity and downstream processing of the herbal-derived compounds, such compounds are gaining importance in the modern drug discovery process due to advancement in various research and developmental strategies in pharmaceutical, synthetic chemistry, combinatorial chemistry and drug design process (Sudhakar *et al.,* 2020; Pallapothu and Sankar, 2021; Sahukari *et al.,* 2021; Ninave and Patil, 2022).

This plant can also be considered a low-cost vegetable with high moisture content (>90%) and high ash value (>18%) and contains higher mineral content such as iron followed by copper, zinc and other micro-elements (Sudhakar *et al.,* 2020; Murugan *et al.,* 2018). The most potential therapeutic treatments are as anticancer (Sanseera *et al,* 2012; Wang *et al.,* 2017), analgesic (Rahman *et al.,* 2010), anti-inflammatory (Rahaman *et al.,* 2010; Soruba *et al.,* 2015; Sahukari *et al.,* 2021), anthelmintic (Chengaiah *et al.,* 2009), antibacterial (Batubara *et al.,* 2016), antifungal (Sakthi *et al.,* 2011; Sherifat *et al.,* 2021), antiviral (Ali *et al.,* 1996), anti-diabetes (Nandhakumar *et al.,* 2009; Junaedi *et al.,* 2014), antioxidant (Ruslan *et al.,* 2015), antiulcer (Kalimuthu *et al.,* 2010), anti-ageing (Husniyah, 2018), anti- hyperlipidemic (Nandhakumar *et al.,* 2009), anti-hemolytic, anti-obesity(Naik *et al.,* 2019; Moon *et al.,* 2013), anti-venom(Alam *et al.,* 1998), hepatoprotective (Globin med, Malaysian journal) and the hypothesis of the study conducted by Reddy JS *et al.* titled "Wound healing effects of *Heliotropium indicum, Plumbago zeylanicum* and *Acalypha indica* in rats" is that the three *plants H. indicum, P. zeylanicum,* and *A. indica* have potential wound healing properties, and their application would result in a significant improvement in the rate and quality of wound healing in rats by evaluating the efficacy of these plants in promoting wound healing properties (Reddy *et al.,* 2002). (Chekuri et al., 2020; Acalypha Indica L – GlobinMed, 2022)

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## Materials And Method

### Collection and Processing of Plant Sample

The plant sample (*A. indica*) was collected from the Medchal district of Hyderabad, Telangana state during the month of January. The plant sample was packed in zipper pouches and bought to the laboratory facility. The plant sample was cleaned by washing with double distilled water followed by washing with 10% sodium chloride solution and again washed with double distilled water. The different plant parts were separated and kept for drying in shade for 10–15 days to reduce the moisture content. After drying, the plant samples were milled into powder form and then sieved by a particular mesh size to get uniform particle size. The dried powdered plant material was then stored in an air-tight container for further analysis (Chandel *et al.,* 2011).

### Plant Extract Preparation

The dried plant powder was put separately in 100% acetone and Hydro-alcohol (60-part water & 40-part ethyl alcohol) in the ratio of 1gm plant sample in 20 mL of extraction solvent.

The sample solvent mixture was then kept in amber colored sample bottle with mild shaking for 76 hours. Afterward, The mixture was then filtered and collected for experimental analysis (Altemimi *et al.,* 2017).

### Physiochemical Analysis of Plant-derived Extracts

*Extractive Value*

The extractive value indicates the efficiency of extraction of the crude drug from the plant sample (Chandel *et al.,* 2011). This indicator was performed for both the organic solvent extracts (Ajazuddin, 2010).

*Qualitative screening*

The various plant metabolites that are present in the solvent extracts such as alkaloids, flavonoids, phenolics, carbohydrates, proteins and amino acids, Quinone, terpenoids etc. (Usman *et al.,* 2009; Pant *et al.,* 2017) were characterized by various chemical methodologies as described by Trease and Evans (1989) that determined the presence or absence of such metabolites in the plant material.

### Quantitative Estimation of Flavonoids and Phenolics Compounds

The total flavonoid content was estimated by the standard Aluminium chloride method (da Silva *et al.,* 2015; Sankhalkar *et al.,* 2016) the and total phenolic content was estimated by the Folin-ciocalteue method (Khodaieet *et al.,* 2012; Sankhalkar *et al.,* 2016).

### In Vitro Assay for Plant-derived Extracts

The plant extracts were then screened for their different in vitro pharmacological activities such as antibacterial and antioxidant activity.

Anti-microbial studies (Balouiri *et al.*, 2016) against different pathogenic bacteria such as *E. coli, Staphylococcus sp., Pseudomonas sp., Salmonella sp.* and *Bacillus sp.* was done by agar well diffusion method where the presence of clear zone around the wells (no growth of microorganism) indicate that the test microorganism is susceptible to the plant extract whereas the absence of clear zone indicates that the test microorganism is resistance to the plant extract.

Antioxidant activities were studied by DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity (Blois, 1958; Brand-Williams *et al.,* 1995) using Ascorbic acid as standard. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method has been widely used for antioxidant study of plant extracts. The DPPH molecule is highly stable and possesses maximum absorbance at 517 nm. The antioxidant molecule can reduce the DPPH molecule. The percentage of scavenging activity is calculated as follows:

$$Percentage\ of\ scavenging\ activity = \frac{(Absorbance\ control - Absorbance\ test)}{Absorbance\ control} * 100$$

The anti-inflammatory activity was studied by Albumin denaturation assay using indomethacin as standard (Dharmadeva *et al.,* 2019). The percentage of denaturation activity is calculated colorimetrically by recording the absorbance of the test sample and blank sample at 68 0nm and calculated by the following equation:

$$Percentage\ of\ denaturation = \frac{(Absorbance\ control - Absorbance\ test)}{Absorbance\ control} * 100$$

The qualitative compound profiling was done by high-performance chromatographic (Normal HPLC) procedure. The constituent fraction was fractioned and separated with different retention times and peak areas. Conditions for HPLC were**:**

Mobile phase: Isocratic type Mobile Phase: Ethanol: Acetone: Formic Acid = 70: 29: 1. Column Type: C18 Flow rate: 1.5 mL/min Injection volume: 5 µL Run Time: 20 minicolumn Temp: 25°C Detector λmax: 260 nm.

### Green Synthesis of Silver Nanoparticles

The leaf extracts were first screened for the synthesis of silver nanoparticles from silver nitrate solution. 1 part of leaf extract and 9 part of 1 mM silver nitrate solution were added, mixed thoroughly, and then heated at 60–80°C for 15–20 minutes. The color of the mixture turns into a reddish brown color that indicates the formation of silver nanoparticles (Prasad *et al.,* 2011; Fox *et al.,* 2020).

Characterization of Silver Nanoparticles: The silver nanoparticles were further characterized by spectral analysis by UV-vis Spectrophotometer (Dubeya *et al.,* 2010) and morphology study by Scanning Electron microscope (Begum *et al.,* 2009; Hassan *et al.,* 2021. Tahir *et al.,* 2022).

## Results

The young, tender and disease-free leaves of *A. indica* plant were selected for the study (Fig. 1). The leaves were collected in the month of October. The leaves were dried in an aseptic condition with minimal contamination, processed into powdered form and then plant extracts (Acetone and Hydroalcoholic) (Fig. 2) were obtained by maceration process (cold extraction process).

**Fig. 1:** Leaves sample of *A. indica.* Dried Powdered leaf of *A. indica*

**Fig. 2:** Solvent extract for Dried Powdered leaf of *A. indica.* Left – Acetone extract (Reddish Brown coloration) and Right-Hydroalcoholic extract (Brownish coloration).

**Table 1:** Phytochemical analysis of aqueous and hydroalcoholic leaf extract of *A. indica*.

| *Test for* | *Test name* | *Positive observation* | *Acetone extract* | *Hydroalcoholic extract* |
|---|---|---|---|---|
| Alkaloid | Wagner's Test | Reddish brown coloration/precipitate | + | ++ |
| Carbohydrate | Molisch Test | Formation of red or dull violet color at the interface of the two layers | ++ | + |
| Glycosides | Keller Killiani test | Brown ring at the interface, Brown-greenish ring may form. | ++ | + |
| Flavonoids | Alkaline reagent test | Intense yellow coloration that becomes colorless on addition of dil.HCl. | + | ++ |
| Phenol | Aq. $FeCl_3$ Test | Deep blue/Black coloration | + | ++ |
| Amino acid | Ninhydrin Test | Formation of purple color. | - | + |
| Saponin | Foam Test | Formation of persistence foam | ++ | + |
| Tannin | Braymer's Test | Formation of blue or greenish color | - | + |
| Terpenoids | Salkowaski Test | Formation of reddish brown precipitate | + | - |
| Quinone | Conc. HCl Test | Formation of yellow precipitate or coloration. | - | + |
| Steroids | Liebermann-Burchard test | Formation of greenish blue color | ++ | - |
| Coumarin | Alkaline test | Formation of yellow color | - | - |
| Resin | Turbidity test | Formation of turbidity | - | - |

The extractive value of a crude drug for acetone extract and hydroalcoholic extract were found to be 3.75 and 4.45%, respectively.

Qualitative Phytochemical analysis revealed that acetonic leaf extract contains different metabolites such as alkaloid, carbohydrate, glycoside, flavonoid, phenol, saponin, terpenoid, and steroid whereas the Hydroalcoholic extract contains alkaloid, carbohydrate, glycoside, flavonoid, phenol, amino acid, saponin, tannin, quinone (Table 1).

The quantitative estimation of Flavonoids and phenolic was established for acetone and hydroalcoholic extract. For quantitative estimation, Quercetin standard curve (Fig. 3) and Tannic acid standard curve (Fig. 4) were established for Flavonoid and Phenol content, respectively.

For estimation of unknown concentration of flavonoid in sample

Slope y= 0.0033x + 0.0659

$R^2 = 0.992$

Where y = Measured absorbance at 415 nm

x = Flavonoid Concentration (equivalent to mM Quercetin)

$$Flavonoid\ content\ (x) = \frac{y - 0.0659}{0.0033}$$

For Quantitative estimation of Flavonoids in the different extracts, Flavonoid content (x) (equivalent mM of Quercetin) was estimated by substituting the value of absorbance at 415 nm (y) in the equation.

For estimation of unknown concentration of phenol in sample

Slope y= 0.0034x + 0.3582

$R^2 = 0.9817$

Where y = Measured absorbance at 765 nm

x = Phenol Concentration (equivalent to mM Tannic acid)

$$Phenol\ content\ (x) = \frac{y - 0.3582}{0.0034}$$

For Quantitative estimation of Phenolic in the different extracts, Phenol content (x) (equivalent mM of Tannic acid) was estimated by substituting the value of absorbance at 765 nm (y) in the equation.

The Flavonoid and Phenolic content was estimated in the leaf extract (acetone and hydroalcoholic) from the slope equation as indicated in their respective standard curve. Higher flavonoid content (84.07 ± 1.44 equivalent to mM of Quercetin) was found in Acetone leaf extract, whereas the Hydroalcoholic leaf extract had higher phenol content of 148.57±2.85 (equivalent to mM of Tannic acid (Tables 2, and 3, Fig. 5).

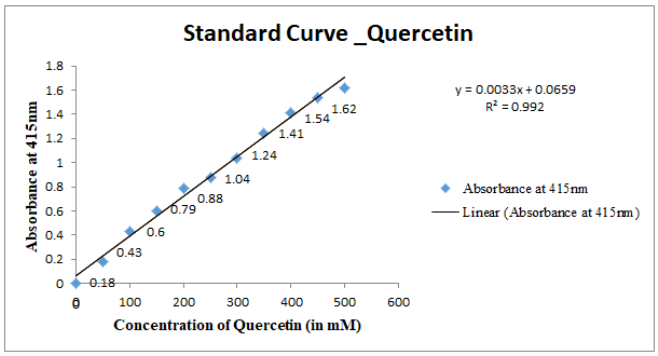



**Fig. 3:** Quercetin Standard curve (For Quantitative estimation of Flavonoids)

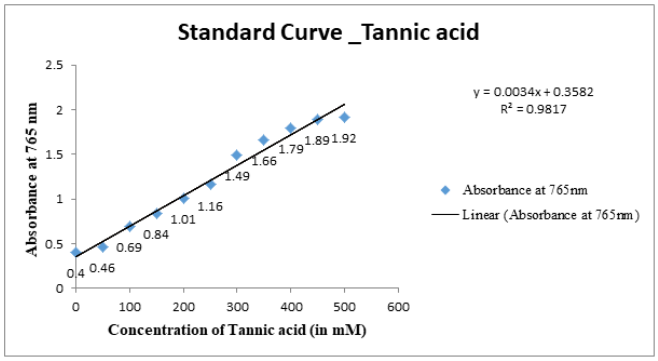



**Fig. 4:** Tannic acid Standard curve (For Quantitative estimation of phenol)

**Table 2:** Flavonoid content in Leaf extracts

| *Sample (leaf extract)* | *Absorbance at 415 nm* | | | *Flavonoid content (equivalent to mM of Quercetin)* | | | *Mean Flavonoid content ± S.D.* |
|---|---|---|---|---|---|---|---|
| Acetone | 0.33 | 0.35 | 0.35 | 80.03 | 86.09 | 86.09 | **84.07 ± 1.44** |
| Hydroalcoholic | 0.28 | 0.27 | 0.28 | 64.87 | 61.84 | 64.87 | **63.86 ± 1.46** |

**Table 3:** Phenol content in Leaf extracts

| *Sample (leaf extract)* | *Absorbance at 765nm* | | | *Phenol content (equivalent to mM of Tannic acid)* | | | *Mean Phenol content ± S.D. (equivalent to mM of Tannic acid)* |
|---|---|---|---|---|---|---|---|
| Acetone | 0.76 | 0.75 | 0.75 | 118.18 | 115.23 | 115.23 | **116.26 ± 2.84** |
| Hydroalcoholic | 0.87 | 0.87 | 0.85 | 150.53 | 150.53 | 144.65 | **148.57 ± 2.15** |

**Table 4:** Antibacterial assessment of acetone and hydroalcoholic leaf extract.

| *Sl. No.* | *Pathogenic Bacteria* | *Zone of inhibition (in mm)Acetone extract* | *Zone of inhibition (in mm Hydroalcoholic extract* |
|---|---|---|---|
| 1. | E. coli | -- | 13 |
| 2. | P. aeruginosa | 32 | 20 |
| 3 | S. aureus | -- | 34 |
| 4 | Salmonella sp. | 03 | -- |
| 5 | Bacillus sp. | 21 | 18 |

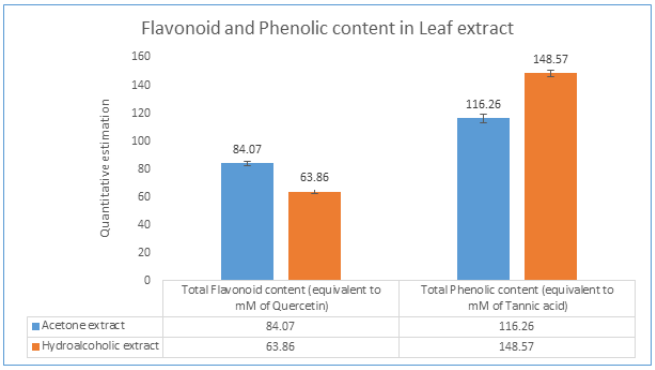



**Fig. 5:** Flavonoid and Phenolic content in the leaf extract.

## In-Vitro Studies

Antibacterial assay: The acetone extract of *A. indica* had better antimicrobial activity against *Pseudomonas sp., Bacillus sp.* and *Salmonella sp.* but didn't show any antibacterial activity against another test organisms such as *Escherichia coli* and *Staphylococcus aureus* whereas in case of hydroalcoholic extract, highest activity was shown against *S. aureus* and also gave antibacterial activity against *Pseudomonas sp., E. coli* and *Bacillus sp.*(Table 4). Compared to acetone extract, a better antibacterial effect was found for Hydroalcoholic extract against test pathogenic bacteria (Fig. 6).

Antioxidant assay: The plant extract was determined for the antioxidant activity by changing the coloration of the standard DPPH solution from purple to yellow. The antioxidant property was determined quantitatively by calculating the percentage of inhibition. Higher antioxidant activity was observed in Acetone leaf extract as compared to Hydroalcoholic leaf extract of *A. indica* (Table 5; Fig. 7).

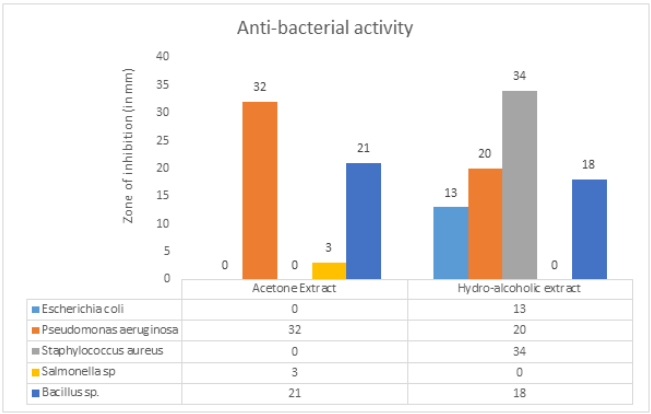



**Fig. 6:** Antibacterial assessment of acetone and hydroalcoholic leaf extract.

**Table 5:** Antioxidant study (DPPH method) for Aqueous and Hydroalcoholic leaf extract of *A. indica*.

| *Sl.No.* | *Samples* | *Test (in triplicates)* | *Absorbance at 517 nm* | *Radical Scavenging activity* | *Mean Radical Scavenging activity ± S.D.* |
|---|---|---|---|---|---|
| 1. | Blank | - | 0.47 | - | - |
| 2. | Ascorbic Acid (0.1 mg/mL) | - | 0.12 | 74.46 | **74.46** |
| 2. | Acetone Extract (0.1 mg/mL) | 1/3 | 0.23 | 51.06 | **51.74 ± 1.71** |
| | | 2/3 | 0.22 | 53.10 | |
| | | 3/3 | 0.23 | 51.06 | |
| 3. | Hydroalcoholic Extract (0.1 mg/mL) | 1/3 | 0.36 | 23.40 | **21.98 ± 1.34** |
| | | 2/3 | 0.37 | 21.27 | |
| | | 3/3 | 0.37 | 21.27 | |

**Table 6:** Anti-inflammatory study (Albumin denaturation assay) for aqueous and hydroalcoholic leaf extract of *A. indica.*

| *Sl.No.* | *Samples* | *Test (in triplicates)* | *Absorbance at 680 nm* | *Anti-inflammatory* | *Mean anti-inflammatory activity ± S.D.* |
|---|---|---|---|---|---|
| 1. | Blank | - | 0.54 | - | - |
| 2. | Indomethacin (0.1 mg/mL) | - | 0.46 | 14.81 | **14.81** |
| 2. | Acetone Extract (0.1 mg/mL) | 1/3 | 0.57 | - | - |
| | | 2/3 | 0.55 | - | |
| | | 3/3 | 0.56 | - | |
| 3. | Hydroalcoholic Extract (0.1 mg/mL) | 1/3 | 0.42 | 22.22 | **22.22 ± 2.34** |
| | | 2/3 | 0.41 | 24.07 | |
| | | 3/3 | 0.43 | 20.37 | |

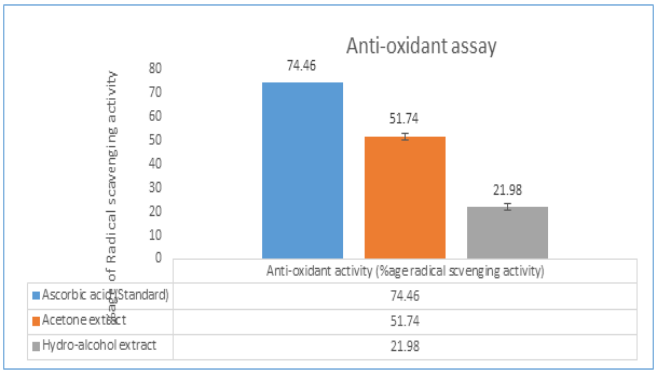



**Fig. 7:** Antioxidant study (DPPH method) for Aqueous and hydroalcoholic leaf extract of *A. indica.*

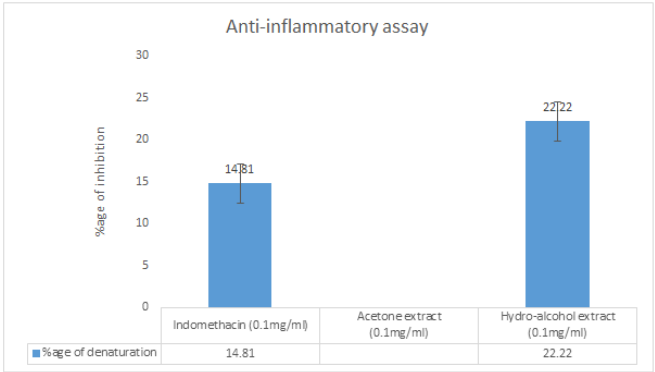



**Fig. 8:** Anti-inflammatory study (Albumin denaturation method) for Aqueous and Hydroalcoholic leaf extract of *A. indica.*

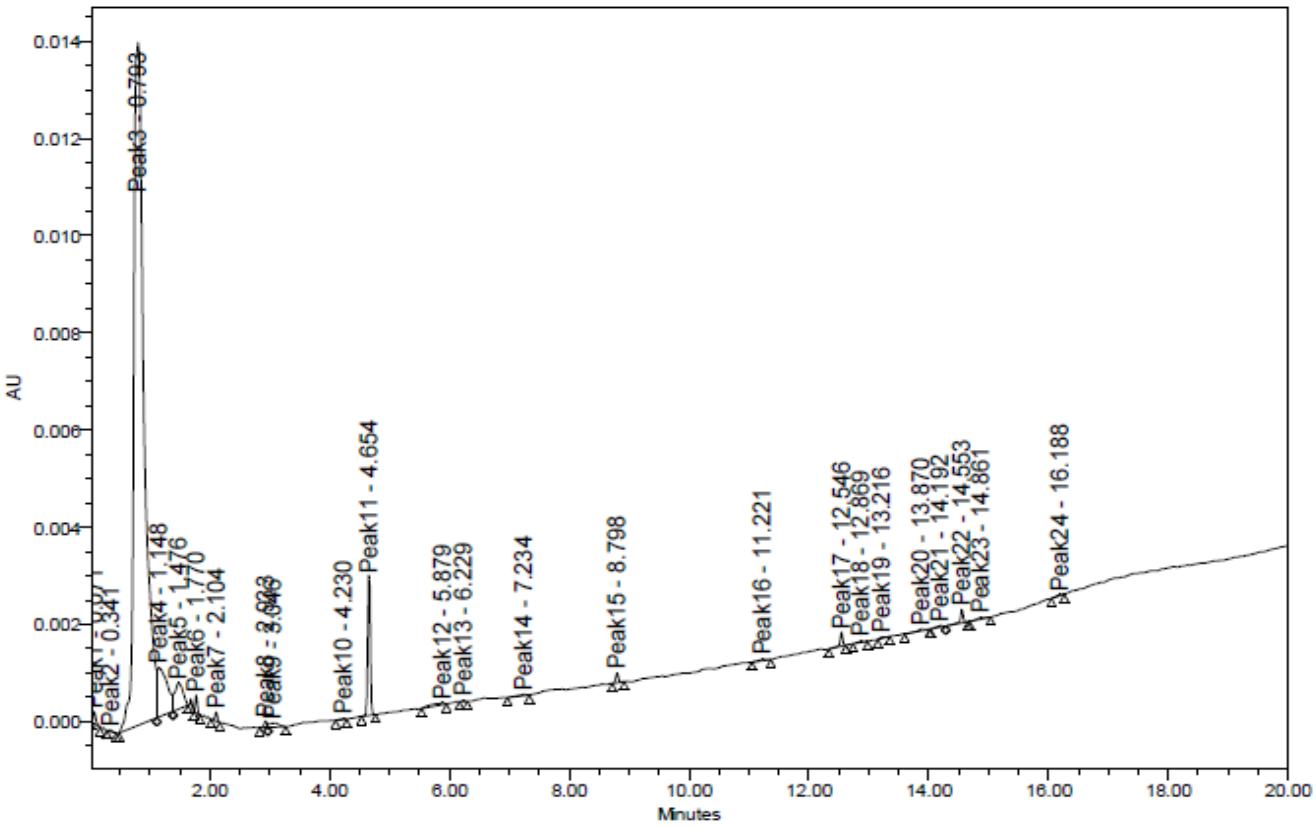
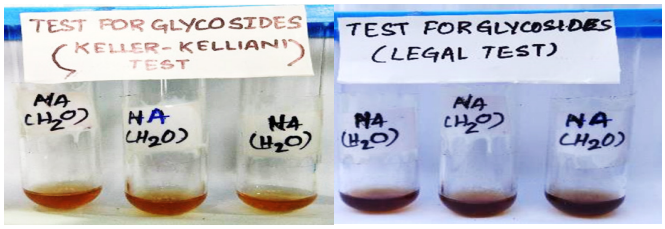



**Fig. 9:** HPLC-Chromatogram for Hydroalcoholic extract.




Silver nitrate solution and Hydroalcoholic Leaf extract, at Time = $T_0$

Colour change into reddish brown coloration indicate the formation of silver nanoparticles

**Fig. 10:** Screening for leaf extract for formation of silver nanoparticles from silver nitrate solution.

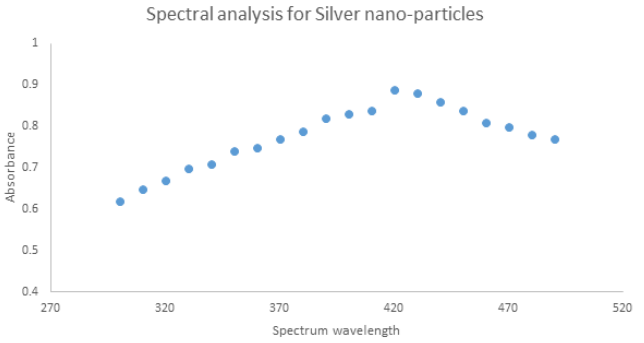



**Fig. 11:** Spectral analysis for production of silver nanoparticles by UV-visible Spectrophotometer.

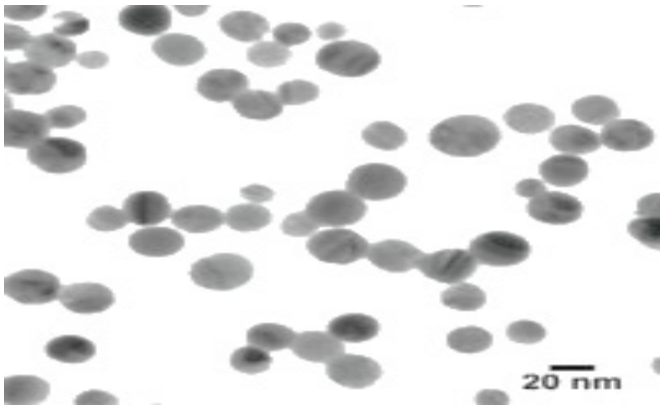



**Fig. 12:** Assemblies were found to be aggregated of Silver nanoparticle (Ag-NP) synthesized by Hydroalcoholic Leaf extract.

Anti-inflammatory activity: The anti-inflammatory effect can be studied by the extract's capability to inhibit protein denaturation. The hydroalcoholic extract gave greater activity as compared to the standard indomethacin, whereas the acetone extract didn't have any such activities (Table 6; Fig. 8).

As described in methodology, qualitative profiling by HPLC analysis for the hydro-alcohol extract at standard experimental conditions revealed the presence of 24 different compounds as comparable at different retention times (Fig. 9).

The plant extracts were screened for the production of silver nanoparticles from molecular silver nitrate solution. The hydroalcoholic extract produced silver nanoparticles that were screened by change in coloration of the solution into reddish brown coloration (Fig.10).

Silver nanoparticle characterization was proposed with absorbance peaks observed in the spectral analysis from 420 to 450 nm that corresponds to the formation of Silver nanoparticles (Fig. 11).

The silver nanoparticles thus produced were purified by repeated centrifugation. After each centrifugation cycle, the pellet was collected, re-suspended in distilled water, centrifuged again, and repeated for 5 to 10 cycles. The purifies silver nanoparticles were analyzed for their morphology by Transmission Electron Microscope. The morphology of the synthesized Silver nanoparticles are highly variable. The assemblies were found to be aggregated of Silver nanoparticles (Ag-NP) in the range 15 to 20 nm (Fig. 12).

## Conclusion

The plant of *A. indica* has been used in conventional medicine and traditional medicinal practices based on phytochemical analysis of different extracts and their various types of biological and therapeutic activities such as antibacterial and anti-inflammatory properties. Besides, the plant extract was found to have antioxidant properties.

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## Author's Contribution

The Project was conceptualized and carried out by Koppula Prawan under the guidance and supervision of Dr. Srilatha Reddy Gantala. While Shilpa Kalukuri, Wilcina Genevieve Dommat, Vaishnavi Volukula, Srijitha Gangi and Varshitha Saval assisted in sample collection and data interpretation.

## Conflict of Interest

None

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