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A STUDY ON PHYTOCHEMISTRY OF *CASSIA HIRSUTA*: QUALITATIVE, QUANTITATIVE AND BIOLOGICAL EVALUATION. AN INVITRO STUDY

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ABSTRACT

Long before prehistory, plants were employed as medicine. Medicinal plants provide wide variety of therapeutic phytochemicals is reasonably safe. The phytochemical screening confirmed the presence of alkaloids, carbohydrates and amino acids, flavonoids, polyphenols in the extracts, Quantitative estimation revealed that ethanolic extracts contained higher phenolic content (0.15%) compared to aqueous extracts (0.10%)The ethanolic extract of *Cassia hirsuta* demonstrated antimicrobial action against *Escherichia coli* evident from the observed zone of inhibition. DPPH assay suggested 96.20 % scavenging activity at 1000 ug/ml.IR spectroscopy confirmed the presence of important functional groups such as O–H (alcohols/phenols), C=O (carbonyl compounds), C–O (alcohols/ethers), and C–H (aliphatic groups). These functional groups are characteristic of secondary metabolites with known bioactivities, supporting the phytochemical and biological assay results.

KEYWORDS: *Cassia hirsuta*, DPPH assay, antimicrobial action, IR spectra.

INTRODUCTION

Cassia hirsuta is an evergreen shrubby herb (0.2-2.4 m high), pubescent or sometimes pilose with hairy leaves, that is rarely found in Chittagong especially in the hilly area, although it is widely distributed in Bolivia, Caribbean Jamaica, Cuba, Colombia, Ecuador, El Salvador, Peru and Surinam, Nigeria, east and central Africa. It is also found in some region of India and Myanmar. Different parts of this plant have been used for stomach troubles, dysentery, abscesses, rheumatism, hematuria, fever and other diseases. The root is pasted with cumin and taken internally to treat stomach burning after a meal. The leaves are successfully and commonly used to fix bone fractures and sprains in cattle and in humans. Oral administration of pow- dered dried seeds works against impotency and weakness (Jamir et. al., 2010). Powdered seeds are also used in various dental complications (Revathi and Parimelazhagan, 2010]. In India, 19 species of the genus Cassia, which are useful in various ways to mankind, are well documented and their distribution, identification keys and ethno medical properties are provided.

Fig.1: *Cassia hirsuta.*

MATERIALS AND METHODS

Collection of leaves

The leaves of *Cassia hirsuta* were collected from Hoskote, Bangalore District, Karnataka, India. The collected leaves material was immediately sprayed with ethanol to cease the enzymatic degradation of secondary metabolites. The leaves were shade dried and powdered

inside the laboratory within 10-15 days at room temperature (28-30°C). The shade dried, powdered 16gm leaf material was subjected to soxhlet extraction successively and separately from 95% ethanol and distilled water in a soxhlet extractor for 20 hours each. The extracts were concentrated to dryness in a flash evaporator (Buchi) under reduced pressure and controlled temperature (50-60°C) to obtain the crude extract. The phytochemical screening is done by standard procedures.

QUALITATIVE ANALYSIS

Qualitative analysis of leaf extracts is crucial for identifying the types of chemical compounds present, which helps in understanding the potential medicinal or nutritional value of the plant. The ethanol and water extracts of *Cassia hirsuta* leaves were subjected to standard chemical tests.[1,2,3,5,15,17,22]

QUANTITATIVE ANALYSIS

Determination of total phenolic content

The total phenolic contents of the aqueous and ethanolic extracts were estimated using the Folin Ciocalteau reagent as described by Singleton and Rossi. The calibration curve was plotted by mixing 1 ml aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450 µg/ml Gallic acid solutions with 5.0 ml of Folin Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l).

The absorbance was measured after 30 min at 765 nm. The sample 1 ml was mixed separately with the same reagents, as performed for constructing the calibration curve. After 30 min, the absorbance was measured to determine the total phenolic contents in both extracts separately using the formula,

$$C = C1 \times V/m$$

Where C = total phenolic content in mg/g, in GAE (Gallic acid equivalent), C1 = concentration of Gallic acid established from the calibration curve in mg/ml, V = volume of extract in ml, and m = the weight of the plant extract in gm).[8]

ANTIOXIDANT POTENTIAL

The DPPH ((2,2-diphenyl-1-picrylhydrazyl) assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical

scavengers. DPPH• shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant.

Freshly prepared DPPH solution in 80% methanol was taken in test tubes and Test compounds with different concentrations (31.25, 62.5, 125, 250, 500, 1000ug/ml) were added in 1:1 ratio to all test tubes to achieve the final volume of 1ml. 2. I Test Tubes were incubated for 30mins at RT in the absence of light by covering with aluminium foil. 3. Each and every reaction mixtures keep in separate tubes to keep them as a blank correction. Ascorbic acid with different concentrations was used as standard and 80% Methanol as a Blank. Control sample was prepared containing the same volume without any sample (DPPH alone). The absorbance was read on at 517nm. 6. The % radical scavenging activity of the given compound was calculated using the following formula

% DPPH Radical Scavenging Activity= [(Abs of Control-Abs of Sample)/Abs of Control] × 100

IC_{50} value was calculated from the linear or logarithmic regression equation (Y=mx+ C) derived from % DPPH inhibition graph. In the equation, Y=50, and m and C values were derived from the DPPH inhibition graph.[1,4,5,12,13,14,15,6,17,19,22]

ANTIMICROBIAL ACTIVITY

Bacterial Culture preparation

Cultured organism (Escherichia coli, were centrifuged at 6000rpm for 10 minutes respectively, supernatant was discarded and the pellets were dissolved in 1% (%) Sodium chloride and adjusted to absorbance 1.000 at 600nm under UV spectrophotometer (Genesys 10S UV-VIS Spectrophotometer).

Sample preparation

The sample 10mg (*C.hirsuta* extract) is was dissolved in 1mL. Dimethyl sulfoxide (DMSO) respectively. Different aliquots of the sample and control was prepared by pipetting 10µL (100µg), 20µL (200µg), 30µl. (300µg) and 40µl. (400µg) and the final volume was made upto 50µL by adding DMSO.

Media preparation for MIC

Luria Bertani (LB) agar media (Tryptone 10g, Sodium chloride 10g. Yeast extract 6g, Agar

20g, Distilled water 1000ml) 500mL was prepared by adding Tryptone 5g, Sodium chloride 5g, Yeast extract 3g, Agar 10g, Distilled water 500mL in Erlenmeyer flask and autoclaved at 121°C for 15 mins. The bacterial plates incubated at 37°C for 24h. Later, zone of inhibition was recorded in mm(Millimeter).[1.5,9,18]

IR SPECTROSCOPY

IR spectroscopy provides a powerful tool for characterizing plant extracts, identifying their chemical components, and assessing their quality and potential bioactivity, making it an indispensable technique in plant research and development.

METHODOLOGY

IR spectroscopy is used to identify functional groups in molecules by seeing how they absorb infrared light. The liquid sample of the extract is placed between the sodium chloride plates, while the solid sample is finely ground with dry KBr and compressed into a transparent pellet. The spectrometer is switched on and background spectrum is recorded. The prepared sample is then placed in the sample holder and the spectrum is scanned over the range of 4000-400 cm^{-}. Based n the bands obtained the functional groups present in the extract can be detected.

RESULTS AND DISCUSSIONS

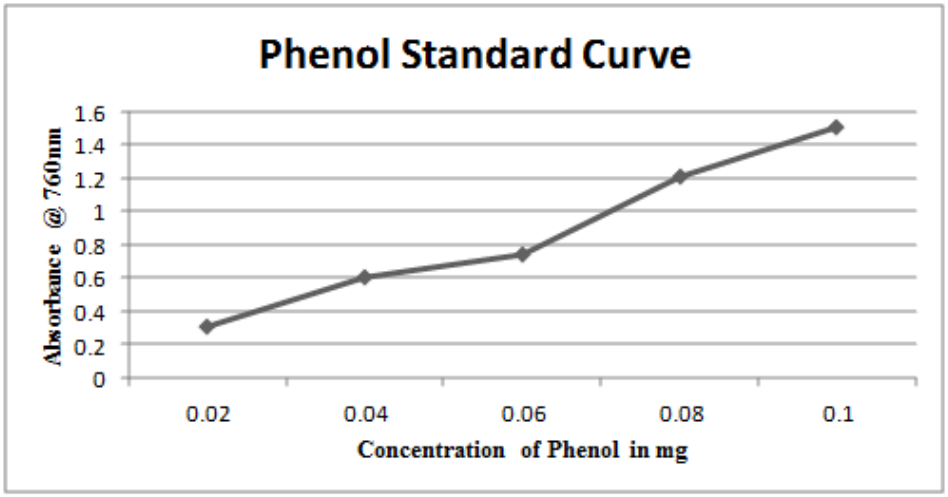
Table 1: Phytochemical screening of ethanol and water extracts.

Sl. No	Phytochemicals tests	Ethanol extract	Water extract
1	Alkaloids	+	+
2	Polyphenols	+	+
3	Tannins	_	_
4	Glycosides	+	-
5	carbohydrates	+	+
6	Saponins	-	+
7	Flavonoids	+	+
8	Steroids	_	-
9	Terpenoids	_	-
10	Proteins and amino acids	+	+

(+) = presence & (-) = absence

Phytochemical screening revealed the presence of alkaloids, polyphenols, glycosides, flavonoids, terpenoids carbohydrates, Proteins and amino acids. However, saponins and tannins were absent in ethanol extract. Water extract revealed the presence of alkaloids, Polyphenols, flavonoids, glycosides, carbohydrates, Proteins and amino acids.

QUANTITATIVE ESTIMATION



Graph 1: Phenol standard curve for the estimation of total phenol content.

Phenolic content report is summarized below:

Table 2: The total phenolic content.

Sl no	Extract	Results (%)
1	Ethanol	0.15
2	Water	0.10

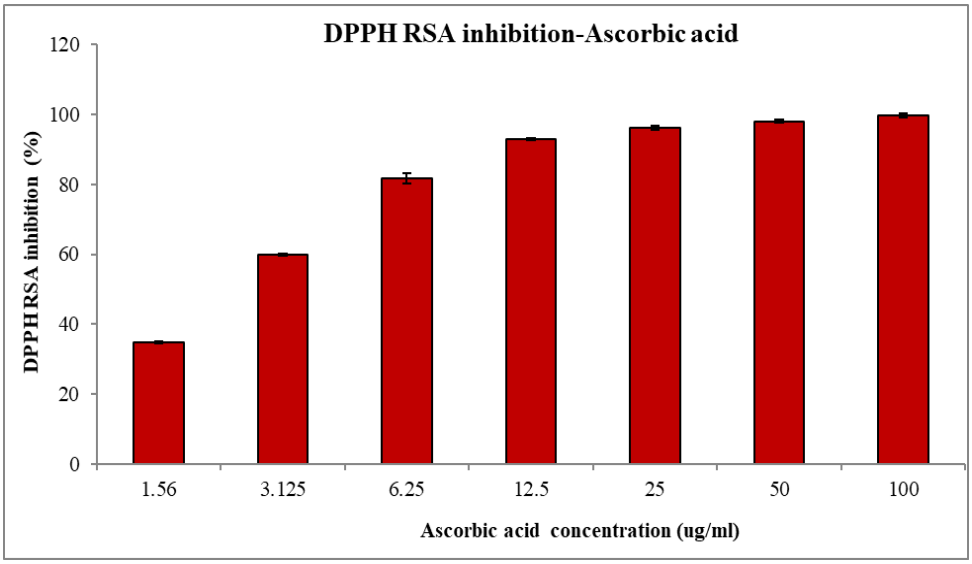
The total phenolic content in the water and ethanol extract of *Cassia hirsuta* leaves was found 0.10% and 0.15% (w/w).

ANTIOXIDANT ACTIVITY

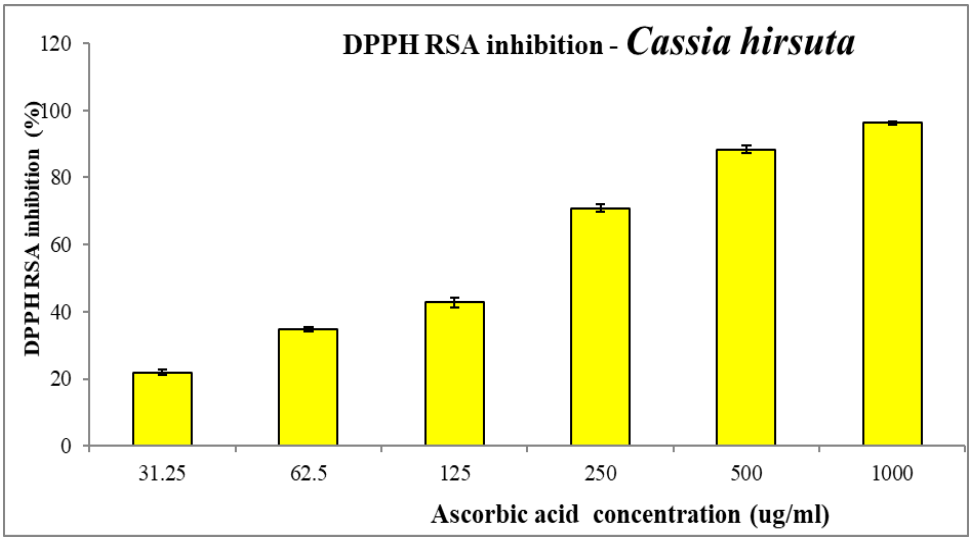
Table 3: DPPH Radical Scavenging Activity (Ethanol extracts).

Sl no	Tested sample	Conc of sample in µg/ml	Percentage Inhibition	IC_{50} (µg/ml)
1	Ascorbic acid	1.56	34.70	2.35
		3.125	59.87	
		6.25	81.79	
		12.50	92.84	
		25	96.11	
		50	98.12	
		100	99.60	
2	*Cassia hirsuta Ethanol extract*	62.5	34.83	182.42
		125	42.69	
		250	70.77	
		500	88.38	
		1000	96.20	

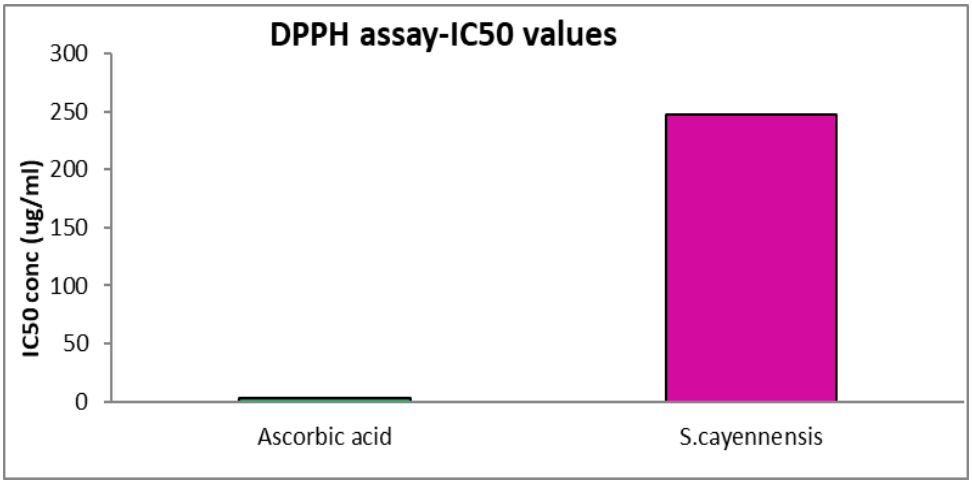
The antioxidant activity determined by free radical scavenging, DPPH assay using gallic acid as standard. Ethanol extract showed 96.20 % scavenging activity at 1000ug/ml.



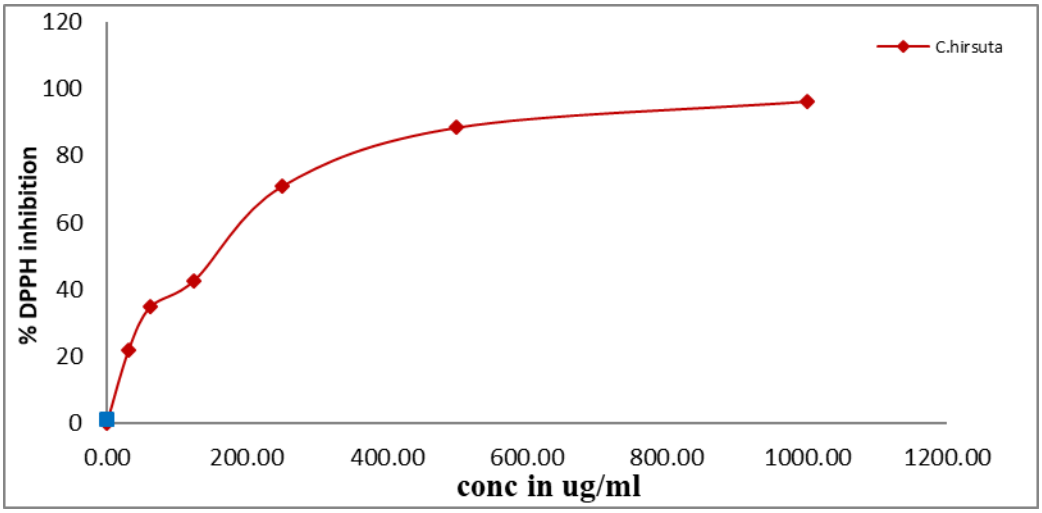
Graph 2: DPPH RSA inhibitory effect of Ascorbic acid with different concentrations.



Graph 3: DPPH RSA inhibitory effect of *Cassia hirsuta* with different concentrations.



Graph 4: IC_{50} value of *Cassia hirsuta* extract and ascorbic acid by DPPH assay.



Graph 5: DPPH Radical Scavenging Activity of Ethanol extracts of *Cassia hirsute.*

ANTIMICROBIAL ACTIVITY

Table 4: Antimicrobial activity of ethanol and water extracts of *Cassia hirsuta*.

Sample	Test strain	Zone of inhibition
ethanol	*Escherichia coli*	observed
water	*Escherichia coli*	Not observed

The present study involved the extraction and analysis of ethanol and water extracts of the plant which exhibited antibacterial activity against *Escherichia coli.* The antibacterial properties may be attributed to the presence of phytochemicals.

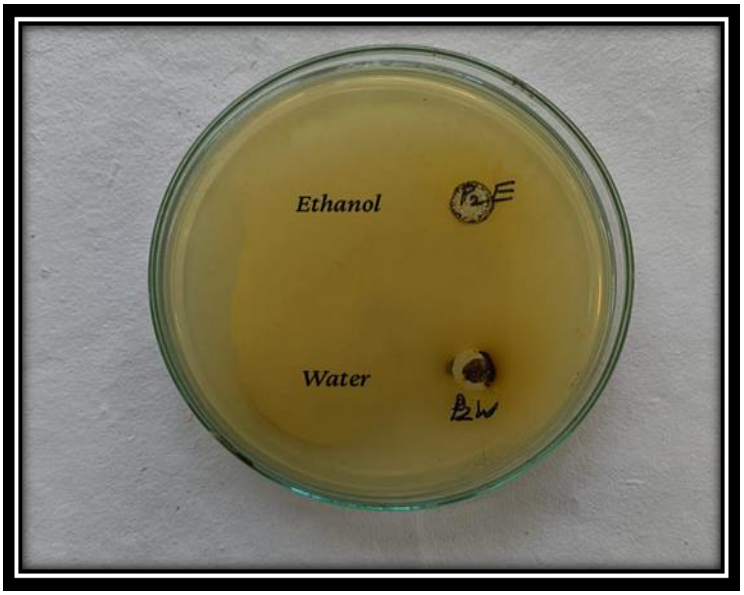
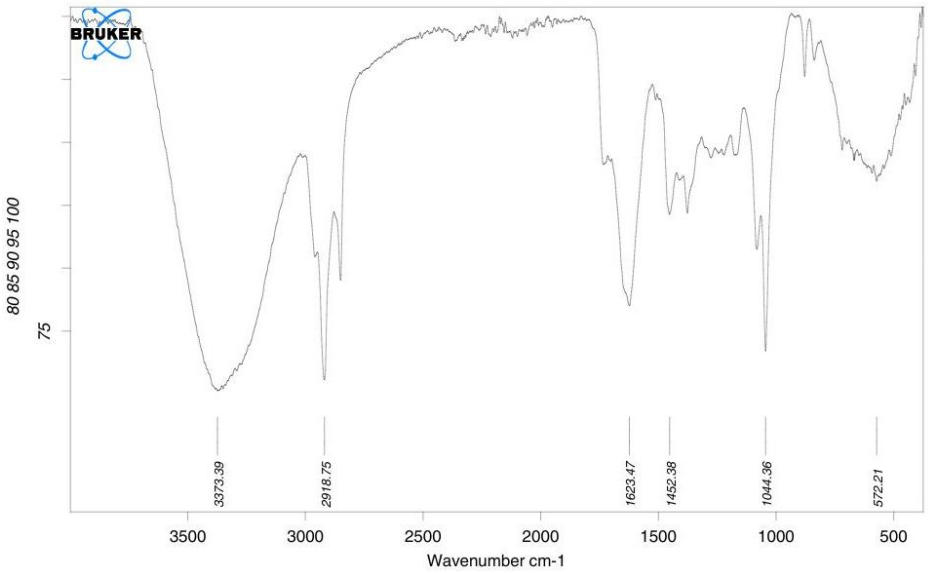


Fig. 2: Zone of inhibition of ethanol and water extract of *Cassia hirsuta* against E.coli.

IR SPECTRA



Graph 6: IR spectra of ethanol extract of *Cassia hirsuta*.

IR Spectrum Interpretation of ethanol extract

DESCRIPTION: ~3373 cm-1

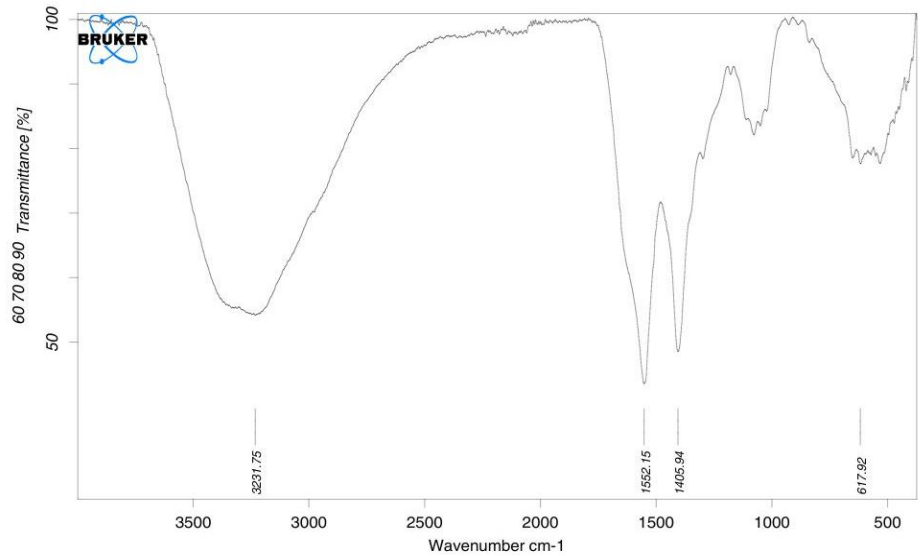
O–H or N–H stretching Broad – likely O–H (alcohol or phenol) or N–H (amide) ~2918 cm-1

C–H stretching Aliphatic C–H (sp^3) groups ~1623 cm-1

C=O stretching Carbonyl group – possibly amide or carboxyl ~1452 cm-1

C–H bending (CH_2, CH_3) Aliphatic groups ~1044 cm-1

C–O stretching Possibly C–O (alcohol, ether, ester) ~572 cm-1 Metal–O or Metal–N Indicative of metal-ligand bond.



Graph 7: IR spectra of water extract of *Cassia hirsuta*.

IR Spectrum Interpretation of water extract

~ Broad Peak near 3300–3400 cm^{-1}:

Indicates: O–H stretching (broad, hydrogen-bonded) or N–H stretching.

Possible group: Alcohol, phenol, or amine. If broad and strong: likely O–H from alcohol or phenol.

If split or sharp: possibly N–H from a primary or secondary amine.~Peaks between 1450–1600 cm^{-1}:Indicates: Aromatic C=C stretching or N–H bending~1375-1450 cm ^-1

Alkane - CH3 (bend) ~ Fingerprint Region (below 1000 cm^{-1}): Contains unique bending vibrations. Often used to confirm identity rather than assign specific functional groups.

CONCLUSION

The qualitative tests confirmed the presence of alkaloids, carbohydrates and amino acids, flavonoids, polyphenols. Quantitative estimation revealed that ethanolic extracts contained higher phenolic content (0.15%) compared to aqueous extracts (0.10%). Since phenolic compounds are known antioxidants, this result highlights ethanol as a more effective solvent for extracting bioactive compounds from *Cassia hirsuta*. The ethanolic extract demonstrated antimicrobial action against Escherichia coli, evident from the observed zone of inhibition. However, aqueous extract showed no inhibitory effect. This suggests that ethanol-soluble phytochemicals, likely phenolics, flavonoids, or tannins, contribute to antimicrobial activity. The DPPH radical scavenging assay revealed moderate antioxidant activity. While standard ascorbic acid exhibited an IC_{50} value of 2.35 µg/ml, the *Cassia hirsuta* extract had an IC_{50} of 182.42 µg/ml. Although comparatively weaker, the extract showed dose-dependent radical scavenging, reaching up to 96.20% inhibition at the highest concentration tested (1000 µg/ml). This indicates that *Cassia hirsuta* possesses appreciable antioxidant capacity, attributable to its phenolic and flavonoid constituents.IR spectroscopy confirmed the presence of important functional groups such as O–H (alcohols/phenols), C=O (carbonyl compounds), C–O (alcohols/ethers), and C–H (aliphatic groups). These functional groups are characteristic of secondary metabolites with known bioactivities, supporting the phytochemical and biological assay results.

However, it is important to note that this study focused primarily on preliminary screening and in vitro assays. While the antimicrobial and antioxidant activities were clearly demonstrated, further work is needed to isolate and characterize the specific active compounds responsible for these effects. Advanced techniques such as HPLC, GC-MS, and

NMR spectroscopy can be employed in future research to identify, purify, and determine the structures of bioactive constituents. Additionally, toxicity studies and in vivo experiments will be essential to establish the safety and efficacy of these compounds for potential therapeutic use.

Conflict of Interest: None.

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