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HPLC CHARACTERIZATION OF PHARMACOLOGICALLY ACTIVE METABOLITES FROM CASSIA AUGUSTIFOLIA LEAF EXTRACT

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ABSTRACT

Cassia angustifolia also referred to as senna makkai or cassia senna, is a plant native to Saudi Arabia, Egypt, and Yemen, and is widely cultivated in India. This medicinal herb has been traditionally utilized to address various ailments, including liver diseases, constipation, typhoid, and cholera. This investigation aimed to assess the in-vitro antimicrobial, antioxidant, and anticancer activities, as well as the phytochemical components, of both aqueous and organic extracts derived from the leaves of C. angustifolia. The antimicrobial activities of C. angustifolia aqueous and organic extracts (methanol, ethanol, acetone, ethyl acetate) were examined using the disk diffusion method. The bioactivity-guided screening of C. angustifolia extracts resulted in the isolation and identification of three flavonoids: quercimeritrin (1), scutellarein (2), and rutin (3), which are reported for the first time from this plant. Furthermore, extracts of C. angustifolia demonstrate the ability to impede microbial growth.

Keywords: Cassia Angustifolia, Antimicrobial, Metabolites, Quercimeritin, Scutellarein, Rutin

1. INTRODUCTION

In recent years, there has been a concerning rise in antibiotic resistance among several human pathogenic bacterial and fungal strains, which contributes to the recurrence of infectious disorders. Urinary tract, circulation, and respiratory organs are prevalent sites of infections caused by antibiotic-resistant bacteria globally (1). Multidrug-resistant bacterial and fungal strains are the primary contributors to hospital-acquired infections, diminishing drug efficacy and ultimately leading to treatment failure (2). This circumstance has necessitated the discovery of more efficacious pharmaceuticals.

Microorganisms have been the principal source of antibiotics, and with the growing adoption of herbal remedies, the evaluation of medicinal plants for new active chemicals has emerged as a significant source of novel antibiotics (3). Comprehensive studies on medicinal plants have shown that they serve as excellent sources of antioxidants [4–6]. These entities engage in activities that scavenge free radicals, playing a crucial role in safeguarding against oxidative stress resulting from the excessive generation of free radicals and reactive oxygen species [7]. These are generated as byproducts of diverse biochemical and physiological processes occurring within the human body [8]. Biomolecular body systems, including lipids, DNA, RNA, and proteins, experience detrimental effects from oxidative stress, ultimately contributing to chronic human diseases such as Alzheimer's, cardiovascular issues, atherosclerosis, cancer, stroke, fibrosis, aging, and diabetes [9].

Numerous studies indicate that the use of medicinal plants, whether as raw extracts or in their chemical forms, is significantly linked to a reduced risk of degenerative diseases related to oxidative stress. This is attributed to their content of antioxidants, including phenolics, flavonoids, vitamins, and carotenoids [10]. Phenolic compounds, including phenolic acids and flavonoids, have been documented to participate in a range of biochemical activities, such as antioxidant, antimicrobial, antithrombotic, antiartherogenic, anti-inflammatory, anti=carcinogenic, and antimutagenic functions [11].

Natural antioxidants derived from plants tend to exhibit greater potency and advantages compared to synthetic alternatives like propylgallate (PG), butylated hydroxytoluene (BHT), t-butylhydroxytoluene (TBH), and butylated hydroxyanisole (BHA) [12]. Reports indicated that synthetic antioxidants contributed to carcinogenesis and liver damage in laboratory animals [13]. Therefore, it is essential to investigate and create natural-origin antioxidants that demonstrate enhanced efficacy and reduced side effects.

C. angustifolia is a conventional medicinal herb from the Caesalpiniaceae family. It is widely recognized as senna makkai or cassia senna. C. angustifolia is indigenous to Saudi Arabia, Egypt, and Yemen. It is a fast-growing shrub, reaching heights of 5–8 meters, widely farmed for its fruit and foliage in the hot, desert regions of India.

This plant is acknowledged in British and American pharmacopoeias [14]. The leaves and pods of C. angustifolia are utilized as a decoction powder for the treatment of intestinal worms, serving as an anti-helminthic. It is extensively utilized as an antipyretic for typhoid, splenic enlargement, cholera, as a laxative, and for conditions such as anemia, toxicity, and genotoxicity induced by Escherichia coli [15].

The global use of C. angustifolia in traditional medicine for a range of health issues, along with existing studies, highlights the need for additional investigation to identify the compounds that contribute to its bioactive properties. This study was carried out to explore the antibacterial, antifungal, antioxidant, and anticancer properties of both aqueous and organic extracts of C. angustifolia.

They underwent phytochemical screening to assess the presence of secondary metabolites and bioactive compounds.

2. METHODS

2.1. FORMULATION OF PLANT CRUDE EXTRACTS

The plant material (whole plant) of Cassia angustifolia was collected, dried and pulverized to form the powder. The plants were collected on the basis of ethno-pharmacological and ethno-botanical literature, Botanical garden, Indira Gandhi Krishi Vishwavidyalaya, Raipur and confirmation of plant material was also done by Dr. P.K. Joshi, Principal Scientist and Team Leader, Centre of Excellence on MAPs and NTFP, IGKVV, Raipur. The collection took place in the flowering season of year 2020-21. The plants were dried in the shade in an open air for 5-10 days and the plant material was ground into fine powder.

For the preparation of test plant material extract modified method by Borde et al. (2016) (16) was adopted. Briefly 20 g portions of the powdered plant material was soaked separately in different solvents for preparation of polar extracts viz. 50% v/v ethanol: distilled water (hydro-alcoholic), distilled water (aqueous), methanol (methanolic) and ethanol (ethanolic) and non-polar extracts (hexane, chloroform and petroleum ether) for 72 h in dark. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) (17). The filtrates obtained were concentrated in vacuo using water bath at 300C.

2.2. DETERMINATION OF INHIBITION ACTIVITY BY DISC DIFFUSION METHOD

A set of bacterial cultures including drug resistant Staphylococcus aureus and Pseudomonas aeruginosa were inoculated into Soyabean casein broth and incubated at 37 0C for 18 h and suspension turbidity was checked as per McFarland standard discussed (appr. 105 CFU/ml). The same procedure was done with test fungal strain followed by the pathogenic isolates viz. Aspergillus niger and Candida albicans. The fungal cultures were inoculated into Sabouraud's dextrose broth separately at 48-72 h. The inhibitory activity of test bacterial strains was determined by the Agar – Disc – Diffusion method (18). The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted Petri plate (19).

2.3. CHARACTERIZATION OF PHARMACOLOGICALLY ACTIVE COMPOUNDS

HPLC analysis was performed in NCS Green Earth Pvt. Ltd., Nagpur, Maharashtra, India using a Shimadzo LC- 2010 HPLC system (Kyoto, Japan), equipped with a Shimadzo LC 2010 UV-VIS detector with a thermo stated flow cell and a selectable two wavelengths of 190 - 370 nm or 371–600 nm. The detector signal was recorded on a Shimadzo LC2010 integrator. The column used was a C-18 block heating-type Shim-pack VP-ODS (4.6 mm interior diameter × 150 mm long) with a particle size of 5 µm. Mobile phase was designed as per the nature of the compound, containing 50 % acetonitrile along with 50 % Phosphate buffer was used at a flow rate of 3.0 ml/min, column temperature 25°C. Injection volume was 40 µl and detection was carried out at specific wavelength having maximum absorbance as calculated by UV absorption spectra at maximum wavelength.

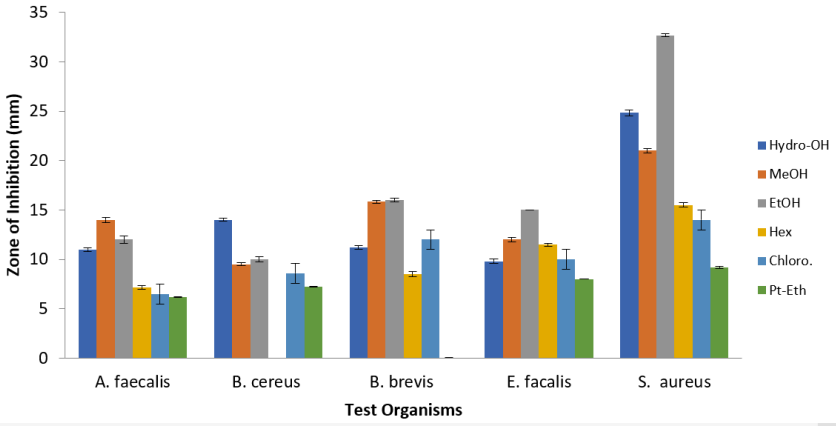
3. RESULTS

3.1. EVALUATION OF ANTIMICROBIAL ACTIVITY

During study antimicrobial activity of various organic extracts of the test plants viz., Cassia angustifolia leaves extracts were determined. It is evident from the data recorded in table that extracts obtained from the test plants showed significant activity against a wide range of bacteria. Antimicrobial potential of the test strains were further determined by different methods. It is evident from data that hydro-alcoholic extract, ethanolic and methanolic extract of Cassia angustifolia leaves showed very strong inhibitory activity against Staphylococcus aureus at 100□g of concentration. It was followed by Pseudomonas aeruginosa, Escherichia coli and B. cereus. Inhibition was significantly declined against Klabsiella pneumoniae, Salmonella typhi and Vibrio cholera at same concentration. Similar trend was also recorded with ethanolic and methanolic fraction against Staphylococcus aureus, Pseudomonas aeruginosa, E. coli and B. cereus while failed to inhibit the growth of Klabsiella pneumoniae. The non-polar solvent's extract of Cassia angustifolia leaves was also showed similar pattern of inhibitory activity against set of test bacterial strains which was maximum against Staphylococcus aureus followed by Pseudomonas aeruginosa while very less effective against gram negative bacteria.

Significant inhibitory activity of Cassia angustifolia plant leaves extracts was also recorded against moldes and yeast during the investigation. Extracts were highly effective against Candida albicans and Aspergillus niger which was followed by S. cervisiae and Schizosaccharomyces sp.

Fig.1. Antimicrobial activity of solvent extracts of *C. angustifolia* leaves on gram positive bacteria through DDM



Tested microorganism	Hydro-alcoholic extract	Methanolic extract	Ethanolic extract	Hexane extract	Chloroform extract	Petroleum ether extract	Ref. (Azithromycin)
Alcaligenes faecalis (MTCC 2763)	11.00±0.15	14.00±0.09	12.00±0.20	7.16±0.02	6.50±0.09	6.16±0.08	35 ± 0.06
B. cereus	14.00±0.02	9.50±0.23	10.00±0.30	ND	8.60±0.05	7.25±0.02	32.1 ± 0.00

(MTCC 633)							
B. brevis *	11.20±0.10	15.83±0.02	16.00±0.30	8.50±0.11	12.0±0.05	ND	33.0 ± 0.01
E. facalis *	9.80±0.04	12.00±0.02	15.00±0.25	11.50±0.04	10.00±0.07	8.00±0.05	29.2 ± 0.8
Staphylococcus aureus (MTCC 187)	24.83±0.05	21.0v0.02	32.67±0.00	15.50±0.20	14.00±0.04	9.16±0.07	26.25±0.02
Escherichia coli (MTCC 1591)	17.16±0.09	20.00±0.07	16.10±0.04	21.00±0.09	15.16±0.33	14.16±0.04	36 ± 0.02
Klabsiella pneumoniae (MTCC 2405)	13.16±0.06	-	10.01±0.05	6.63±0.12	ND	-	30.01±0.02
Pseudomonas aeruginosa (MTCC 779)	27.00±0.14	23.50±0.07	20.00±0.08	25.50±0.30	14.16±0.04	10.25±0.07	34.8 ± 0.07
Salmonella typhi (MTCC 531)	10.16±0.03	-	10.50±0.15	16.33±0.02	ND	-	28.15±0.00
Vibrio cholerae (MTCC 1168)	13.67±0.10	10.0±0.40	10.00±0.09	14.50±0.00	ND	-	26.18±0.20
Tested microorganism	**Hydro-alcoholic extract**	**Methanolic extract**	**Ethanolic extract**	**Hexane extract**	**Chloroform extract**	**Petroleum ether extract**	**Ref. (Flucanazol)**
Aspergillus niger	15.50±0.05	13.16±0.23	20.00±0.30	13.16±0.05	10.33±0.10	ND	26.18±0.00
Candida albicans	12.00±0.08	15.00±0.22	10.00±0.21	11.00±0.26	10.00±0.07	-	25.00±0.01
Saccharomyces cerevisiae MTCC1732	9.50±0.12	9.20±0.31	ND	12.16±0.40	-	-	24.58±0.33
Schizosaccharomyces sp.*	9.00±0.01	ND	9.16±0.21	-	ND	ND	26.00±0.20

- Data are multiple of three observations
- Values ± standard error (SEM)
- DDM: Disc diffusion method
- ND: Not detectable, -: No activity, NT: Not tested

HPLC analysis

Column purified fractions having continuous activity were pooled and further subjected with high performance liquid chromatography. Different peaks received for different parts of Cassia angustifolia extracts which was about 3 and 2 for Hydro-alcoholic extract of Cassia angustifolia leaves (HACAL-1)) HPLC-MS revealed the presence of three bioactive compounds: quercimeritrin (1), scutellarein (2), and rutin (3).

Quercimeritrin (1)

C21H20O12, yellow amorphous powder; 1 H NMR (400 MHz, CD3OD) δ: 3.48-4.60 (6H, m, H-2", H-3", H-4", Ha-5", Hb-5"), 5.25 (1H, d, J = 8 Hz, H-1"), 6.25 (1H, d, J = 2 Hz, H-8), 6.48 (1H, d, J = 2 Hz, H-6), 6.86 (1H, d, J = 8.5 Hz, H-5'), 7.63 (1H, dd, J = 8.5, 2.5 Hz, H-6'), 7.67 (1H, d, J = 2.0 Hz, H-2'). ESIMS m/z 464.38 [M]+, 465.38 [M + H]+ , 487.38 [M + Na] + , 463.37 [M-H]− .

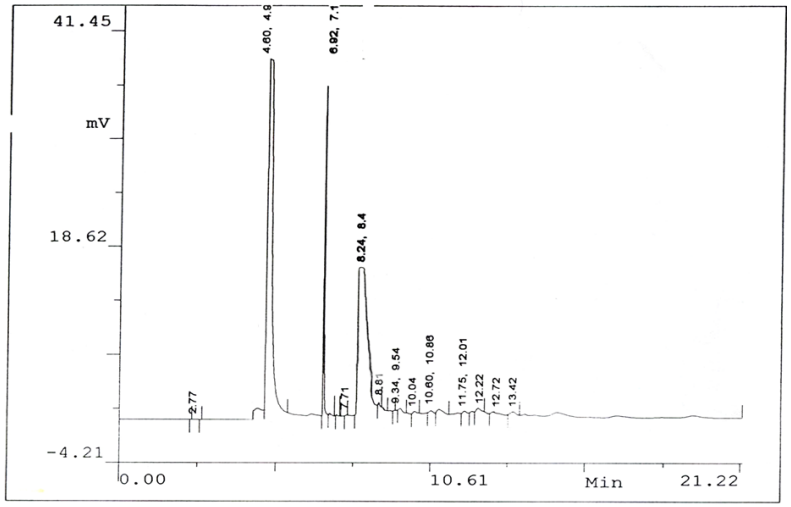
- Data are multiple of three observations
- Value ± SEM
- -: no inhibition
- ND: Not detectable

Scutellarein (2)

C15H10O6, reddish-brown crystals; 1 H NMR (400 MHz, CD3OD) δ: 6.92 (1H, s, H-3), 6.76 (2H, d, J = 8, H-3',5'), 7.09 (2H, d, J = 8, H-2',6'), 7.11 (1H, s, H-8). ESIMS m/ z 286.15 [M]+ , 287.15 [M + H]+ , 309.15 [M + Na] + , 285.14 [M-H]− .

Rutin (3)

C27H30O16, yellowish-brown crytals; 1 H NMR (400 MHz, CD3OD) δ: 8.24 (3H, d, J = 6, H-6'''), 3.27 (1H, m, H-4'''), 3.45 (1H, m, H-5'''), 3.56 (1H, dd, J = 9.5/3.5 Hz, H-3'''),



4. DISCUSSION

Medicinal plants serve as significant sources of biologically active natural products, which have been extensively studied for their curative properties over many years [20, 21]. This study aimed to investigate the bactericidal potential of extracts from C. angustifolia. Majority of the test bacterial and fungal strains are associated with the development of several infectious diseases [22]. The methanol extract of C. angustifolia exhibited the most significant activity among the extracts, demonstrating a wide range of effectiveness against pathogenic bacterial strains. The bactericidal activity has been attributed to the flavonoids present in the methanol extract [28]. Research indicates that methanol and ethanol extracts of C. angustifolia exhibit notable antibacterial activity against E. coli, Klebsiella pneumoniae, and Shigella shinga [23]. The present findings indicate that methanol, ethanol, and ethyl acetate extracts are abundant in flavonoids, which play a crucial role in antimicrobial activities. Flavonoids such as rutin (3) have been documented to exhibit antimicrobial activities against resistant bacterial strains [24].

5. CONCLUSION

This study aimed to investigate the antimicrobial, antioxidant, and anticancer properties of C. angustifolia. The highest levels of antimicrobial activities were noted in the methanol, ethanol, and ethyl acetate extracts, which correlated with the contents of phenolic and flavonoid compounds. The bioactivity-guided screening of methanol, ethanol, and ethyl acetate extracts identified the presence of quercimeritrin (1), scutellarein (2), and rutin (3). These compounds are recognized for their beneficial bioactivities, which encompass antimicrobial, antioxidant, and anticancer properties. The findings highlight the significance of evaluating medicinal plants for their potential antimicrobial, anticancer, and antioxidant properties in addressing resistant bacterial strains, and degenerative diseases linked to oxidative stress.

CONFLICT OF INTERESTS

None.

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None.

REFERENCES

Sharma R, Sharma CL, Kapoor B. Antibacterial resistance: current problems and possible solutions. Indian J Med Sci. 2005; 59:120–9.

Djeussi ED, Noumedem AKJ, Seukep AJ, Fankam GA, Voukeng KI, Tankeo BS, Nkuete HLA, Kuete V. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complement Altern Med. 2013; 13:1–8.

Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. BMC Complement Altern Med. 2008;8:53–7

Ashraf A, Sarfraz RA, Rashid MA, Shahid M. Antioxidant, antimicrobial, antitumor, and cytotoxic activities of an important medicinal plant (Euphorbia royleana) from Pakistan. J. Food Drug Anal. 2014; 23(1):109–15.

Ashraf A, Sarfraz RA, Mahmood A, Din MU. Chemical composition and in vitro antioxidant and antitumor activities of Eucalyptus camaldulensis Dehn. Leaves. Ind Crop Prod. 2015; 74:241–8.

Qayyum A, Sarfraz RA, Ashraf A, Adil S. Phenolic composition and biological (anti diabetic and antioxidant) activities of different solvent extracts of an endemic plant (Helitropium strigosum). J Chil Chem Soc. 2016;61(1):2642–5

Saeed N, Khan RM, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 2012;12:221–33.

Aslanturk OS, Celik TA. Antioxidant, cytotoxic and apoptotic activities of extracts from medicinal plant Euphorbia platyphyllos L. J Med Plants Res. 2013;7:1293–304.

Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with cancer. Life Sci. 2004;74:2157–84.

Art IC, Hollman PC. Polyphenols and disease risk in epidemiological studies. Am J Clin Nutr. 2005;81:317–25.

Alpinar K, Ozyurek M, Kolak U, Guclu K, Aras C, Altun M, Celik SE, Berker KI, Bektasoglu B, Apak R. Antioxidant Capacities of some food plants wildly grown in ayvalik of turkey. Food Sci Tech Res. 2009;15:59–64.

Sherwin FR. Antioxidant. In: Branen R, editor. Food additive biological properties. New York: Marcel Dekker; 1990.

Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, Bedi YS, Taneja SC, Bhat HK. Medicinal plants and cancer chemoprevention. Curr Drug Metab. 2007;9:581

Murray PR, Baroon EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of clinical microbiology. 6th ed. Washington DC: American Society for Microbiology; 1995.

Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plants Res. 2010;4:104–11.

Borde, M. K., Suman, R. K., Mohanty, I. R., & Deshmukh, Y. A. (2016). Dipeptidyl peptidase-IV inhibitory activities of medicinal plants: Terminalia arjuna, Commiphora mukul, Gymnema sylvestre, Morinda citrifolia, Emblica officinalis. Asian Journal of Pharmaceutical and Clinical Research, 180-182.

Mathur, M. L., Gaur, J., Sharma, R., & Haldiya, K. R. (2011). Antidiabetic properties of a spice plant Nigella sativa. Journal of Endocrinology and Metabolism, 1(1), 1-8.

Dulger B, Dulger G. Antimicrobial activity of the leaves of Ballota acetabulosa on microorganisms isolated from urinary tract infections. Turk J Pharm Sci. 2012;9(3):257–262.

Zapantis, A., Lacy, M. K., Horvat, R. T., Grauer, D., Barnes, B. J., O'Neal, B., & Couldry, R. (2005). Nationwide antibiogram analysis using NCCLS M39-A guidelines. Journal of clinical microbiology, 43(6), 2629-2634.

Ahmed N, Mahmood A, Ashraf A, Bano A, Tahir SS, Mahmood A. Ethnopharmacological relevance of indigenous medicinal plants from district Bahawalnagar, Punjab, Pakistan. J Ethnopharmacol. 2015; 175:109–23.

Sanches IS, Saraiva ZC, Tendeir TC, Serra JM, Dias DC, Delencastre H. Extensive Intra-Hospital spread of methicillin resistant Staphyloccocal clone. Int J Infect Dis. 1998; 3:26–31.

Priya B, Anil KS. Anti-cancer potential of flavonoids: recent trends and future perspectives. Biotech. 2013; 3:439–59.

Khan NA, Srivastava A. Antifungal activity of bioactive triterpenoid saponin from the seeds of Cassia angustifolia. Nat Prod Res. 2009; 23:1128–33.

Siraichi JTG, Felipe DF, Brambilla LZS, Gatto MJ, Terra VA. Antioxidant capacity of the leaf extract obtained from Arrabidaea chica cultivated in southern Brazil. PLoS One. 2013; 8:e72733.