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Phytochemical determination and evaluation of antioxidant and antibacterial potential of leaves of *Cassia tora* Linn. and *Ruellia patula*

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Abstract

Primary metabolites, including total soluble sugars, proteins, and lipids, are found abundantly in both plant species, with higher levels observed in *C. tora* compared to *R. patula*. Although primarily associated with plant growth and development, these metabolites play crucial roles in stress tolerance and metabolic regulation. Secondary metabolites, such as total phenols, flavonoids, alkaloids, tannins, and terpenoids, are detected in both species, with *C. tora* exhibiting higher phenolic content and R. patula showcasing elevated levels of flavonoids, alkaloids, and tannins. These compounds fortify plant immunity and have been commercially exploited for their therapeutic potential against oxidative and inflammatory conditions. Extracts from the leaves of *C. tora* and *R. patula* exhibit potent antioxidant activity, with R. patula demonstrating higher efficacy attributed to its richer secondary metabolite profile. Moreover, these extracts display significant antimicrobial properties against both Gram-positive and Gram-negative bacteria. *C. tora* shows higher activity against *E. coli,* while *R. patula* exhibits superior efficacy against *B. subtilis* and *P. aeruginosa.*

The antimicrobial action is attributed to the presence of bioactive secondary metabolites, including alkaloids, saponins, tannins, flavonoids, phenolic compounds, and terpenoids, which disrupt bacterial growth and virulence through various mechanisms. The findings underscore the remarkable antioxidant and antibacterial potential of *C. tora* and *R. patula*, positioning them as "wonder plants" in combating oxidative stress and microbial infections. However, the study's scope is limited to demonstrating the antioxidant and antibacterial effects of the plant extracts, with molecular mechanisms remaining unexplored.

Overall, this research contributes valuable insights into harnessing plant-derived compounds as potential therapeutic agents against microbial threats, paving the way for further exploration in drug discovery and development.

Keywords: Antioxidant potential, antibacterial, primary and secondary metabolites etc

Introduction

The emergence of “Antimicrobial resistance” owing to overuse as well as misuse of the biggest discovery of the 20th century, “the antimicrobial compounds” has shaken the very foundations of the healthcare sector and proved to be a huge setback for the entire medical fraternity as well as scientists across the world. The very tool that was invented to act as a weapon in defending the human race from obnoxious effects of nefarious pathogens has turned into a bane and led to overpowering of disease causing pathogens, thus adding to the miseries of already agonizing situation. Emergence of drug resistant strains characterized by tremendous ability to multiply and reproduce in presence of prevalent antimicrobials has led to narrowing of the pool of available antimicrobials and fueled the need to look for other treatment alternatives with better efficacy. Apart from the developing resistance against antimicrobial, several concerns about the safety of existing antimicrobial is another issue that needs to be addressed. Therefore, the current anarchical situation demands for substitution of the prevalent antimicrobial with safer natural alternatives, with minimum toxicity and equivalent efficacy against the infectious pathogens. In this context, several attempts are being made to unveil an entire range of natural antimicrobial to ameliorate this cataclysm.

Our mother nature has blessed us with pivotal resources in the form of “Plants”, which, apart from bestowing us with the much needed oxygen as well as nutrients for survival; also act as “Emporium of medicinal biocompounds” that enable us to lead a healthy life by evading certain chronic conditions that have been tormenting the human race since immemorial times. Studies over the past few years have strengthened our belief in the practices of traditional medicine described in Ayurveda and Unani medicine, wherein, plants have been described as “powerhouse of nutrients and medicinal compounds”. The ability of plants and their extracts to fight nefarious disease causing pathogens as well as other conditions marked by oxidative stress and inflammatory outburst have been attributed to the presence of bioactive secondary metabolites, namely, phenolics, terpenes, flavonoids, coumarins, saponins and other bioactive phytochemicals **(**Kandar *et al.*, 2021 [19]; Kaur *et al.*, 2021 [20]; Hussein *et al.*, 2019**)** [15].

In this context, the current study is an attempt to study the pharmacological attributes of two medicinal plants, namely, *Cassia tora* (Linn.) Roxb (Caesalpiniaceae), and *Ruellia patula* (Acanthaceae). This has been done by analyzing the content of primary as well as secondary metabolites in leaves of both these plants species followed by an analysis of their antioxidant activity as well as antimicrobial activity against both gram positive as well as gram negative bacteria.

Usage of plant phytochemicals as a part of traditional medicine system has become popular over the last few years, owing to increased efficacy of phytochemicals over the conventional antimicrobials with minimum cytotoxicity and side effects. Moreover, a number of previous studies have shown antimicrobial, anti-inflammatory and antioxidant effects of both these plant extracts in mitigating a number of microbial infections as well as inflammatory disorders (Lee *et al.*, 2013; Das *et al.*, 2010 [5]; Sripriya 2014 [42]; Arulpandi *et al.*, 2011 [3]; Ramadevi *et al.*, 2016 [36]; Lakshmi *et al.*, 2017 [25, 26]; Seenivasan *et al.*, 2023) [41], which led us to further explore the antimicrobial and antioxidant efficacy of leaves of both *Cassia tora* (Linn.) Roxb (Caesalpiniaceae), and *Ruellia patula* (Acanthaceae).

Materials and methods

Selection of plants for further study

On the basis of higher fidelity level and lesser study in the study area, 2 plants were selected for determination of some primary and secondary metabolites and evaluation of their antioxidant and antimicrobial potential. These plants are- leaves of *Cassia tora* (Linn.) Roxb (Caesalpiniaceae), and leaves of *Ruellia patula* (Acanthaceae).

Determination of primary metabolites

The total amount of soluble carbohydrates was calculated using the methodology outlined by Hedge and Hofreiter (1962) [14]. The amount of protein was evaluated using an approach developed by Lowry and colleagues (1957) [29]. For Lipids, method of Jayaraman, 1981 [17] was used.

Determination of secondary metabolites

The spectrophotometric approach was utilised in order to ascertain the level of phenolics that were present in plant extracts. The Folin-Ciocalteu test was employed as the method for determining the total phenol content of the sample (Rasool *et al*., 2011) [38]. The aluminium chloride colorimetric test was utilised in order to determine the total flavonoid concentration (Kaviarasan *et al*., 2007) [21]. The overall alkaloid concentration was given in terms of mg of AE per gramme of extract (Fazel *et al*., 2011). The Folin-Ciocalteu method was utilised in order to quantify the tannins. The amount of tannin was measured in milligrammes of gallic acid equivalents per gramme of extract (Marinova *et al*., 2005) [30]. The technique developed by Ferguson in 1956 was used to determine the terpenoids content (Kim *et al*., 2008) [23].

Preparation of plant extracts

The selected plant parts were washed with running tap water and then air-dried. Those were grounded into powder form and extracted in methanol using cold extraction method. The extracts were dried and their different concentrations were prepared in DMSO for biological activity.

Antioxidant potential

For determination of DPPH radical scavenging potential of the extracted samples 1,1-diphenyl 2-picryl-hydrazil (DPPH) method proposed by Alothman *et al*., (2009) [2] was applied. The mixing of 100 µl aliquot form extracts was done in 3.9 ml taken from 0.1 mM DPPH (methanolic) solution. Then blend was subjected to vortex and left for incubation in the dark for 30 min. Its OD was calculated at 515 nm while methanol was used as blank. Linear plot of concentration versus % inhibition was plotted and by this

IC_{50} values were determined. The antioxidant potential of each extract was showed in form of IC_{50} (stated as the quantity of concentration necessary to prevent DPPH radical development by 50%), find out with the help of inhibition curve.

Antibacterial assay

The well diffusion approach was utilised for antibacterial activity (Irobi *et al*., 1994) [16]. Bacterial cultures of *E. coli, S. aureus, B. subtilis and Pseudomonas aeruginosa* were sub-cultured in Nutrient Agar and then placed in an incubator at 37 degrees Celsius for twenty-four hours. After that, the cultures were transferred to petriplates that contained nutritional agar using cotton swabs that had been sterilised. Onto the agar plates, wells with a diameter of 6 millimetres were drilled, and a solution containing between 25, 50, 75 and 100 micrograms per millilitre of extracts was injected into each well. After incubating the plates, the zone of inhibition in each well was determined using the measurement system. The efficiency of extract against the studied bacteria was measured against that of streptomycin at of similar concentrations. The activity index was determined by dividing the inhibition zone of the test sample by the inhibition zone brought on by the antibiotic. The trials were carried out using three separate sets of controls.

Results

Determination of primary metabolites

In the present study, in leaves of *C. tora* and *R. patula*, primary metabolies (total soluble sugars, proteins, and lipids) were determined. Results are shown in table 1 and figure 1. Results revealed that in leaves of *C. tora,* all the selected primary metabolites were higher than *R. patula*. In *C. tora*, TSS, proteins and lipids were found to be 16.77±2.56 mg/g.dw, 29.54±1.86 mg.g.dw, and 19.88±1.35 mg/g.dw respectively. While in *R. patula*, these were 11.16±1.33 mg/g.dw, 21.28±2.44 mg/g.dw and 15.83±0.75 mg.g.dw respectively.

Table 1: Primary metabolites in the selected plants.

Name of plant	Total soluble sugars (mg/g.dw)	proteins (mg/g.dw)	Lipids (mg/g.dw)
C. tora	16.77±2.56	29.54±1.86	19.88±1.35
R. patula	11.16±1.33	21.28±2.44	15.83±0.75

Determination of secondary metabolites

Secondary metabolites (total phenols, total flavonoids, free flavonoids, bound flavonoids, alkaloids, tannins and terpenoids) in leaves of both the selected plants are given in table 2 and Results showed that leaves of both the plants are rich in these tested secondary metabolites. In *C. tora* these metabolites were found to be 9.65±0.55 mg/g.dw, 2.23±0.65 mg/g.dw, 0.86±0.08 mg/g.dw, 1.37±0.76 mg/g.dw, 5.48±0.53 mg/g.dw, 2.66±0.66 mg/g.dw and 1.43±0.33 mg/g.dw respectively while in *R. petula*, these were recorded to be 8.56±1.12 mg/g.dw, 3.55±0.31 mg/g.dw, 1.26±0.33 mg/g.dw, 2.29±0.17 mg/g.dw, 9.23±1.22 mg/g.dw, 2.87±0.25 mg/g.dw and 2.36±0.66 mg/g.dw respectively.

Table 2: Secondary metabolites in the selected plants.

	Total phenols	Flavonoids			alkaloids	tannins	terpenoids
		total	bound	free			
C. tora	9.65±0.55	2.23±0.65	0.86±0.08	1.37±0.76	5.48±0.53	2.66±0.66	1.43±0.33
R. patula	8.56±1.12	3.55±0.31	1.26±0.33	2.29±0.17	9.23±1.22	2.87±0.25	2.36±0.66

Antioxidant potential

Antioxidant potential of methanolic extract of leave of both the selected plants were subjected for evaluation of free radical scavenging activity using DPPH assay. The results of both plants were compared to ascorbic acid (as standard antioxidant compound). Results are shown in Table 3.

Results indicated that both the plants have good antioxidant potential. *C. tora* showed 5.22% to 21.74% free radical scavenging activity at the concentration of 20-100 µg/ml with an IC_{50} value of 244.17 µg/ml. While *R. patula* showed antioxidant potential at the range of 14.33% to 37.78% with a lower IC_{50} value (147.45µg/ml). lower IC_{50} value indicated the presence of higher antioxidant potential. However, these IC_{50} values are higher than standard natural antioxidant ascorbic acid (68.09 µg/ml).

Table 3: Free radical scavenging activity of both the selected plants.

	% free radical scavanging activity						
	20	**40**	**60**	**80**	**100**	**Regression equation**	**IC_{50} (µg/ml)**
C. tora	5.224787	9.963548	12.7582	16.76792	21.7497	Y=0.1993x+1.3366	244.1716
R. patula	14.33779	21.62819	25.75942	29.89064	37.78858	Y=0.2758x+9.3317	147.4558
ascorbic acid	32.6853	43.49939	51.27582	53.21993	57.35115	Y=0.2953x+29.891	68.09685

Antimicrobial activity

Antibacterial activity was observed against 4 bacteria- *E. coli, S. aureus, B. subttilis* and *P. aeruginosa*. Results are shown in table 4 and figure 1. results indicated that methanolic extracts of both plants showed good antimicrobial potential against all the selected bacteria.

Table 4: Antibacterial activity of the selected plant parts.

Name of samples	**Concentration of samples**							
	25 (µg/ml)		**50 (µg/ml)**		**75 (µg/ml)**		**100 (µg/ml)**	
	IZ (mm)	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**	**IZ (mm)**	**AI**
E. coli								
C. tora	9	0.37	10	0.33	11	0.35	13	0.40
R. patula	7	0.29	8	0.26	10	0.28	11	0.34
Standard	24		30		31		32	
S. aureus								
C. tora	7	0.24	8	0.25	9	0.25	11	0.29
R. patula	7	0.24	8	0.25	10	0.28	11	0.29
Standard	29		31		35		37	
B. subtilis								
C. tora	7	0.22	8	0.22	9	0.23	10	0.24
R. patula	8	0.25	9	0.25	10	0.26	12	0.29
Standard	31		36		38		41	
Pseudomonas aeruginosa								
C. tora	7	0.31	8	0.34	9	0.33	11	0.40
R. patula	8	0.36	9	0.39	10	0.37	12	0.44
Standard	22		23		27		27	

Note: IZ- Inhibition Zone (mm), AI- Activity Index, MIC- Minimum Inhibitory Concentration (mg/L), S- Standard antimicrobial drug (positive control), NA- No Activity.

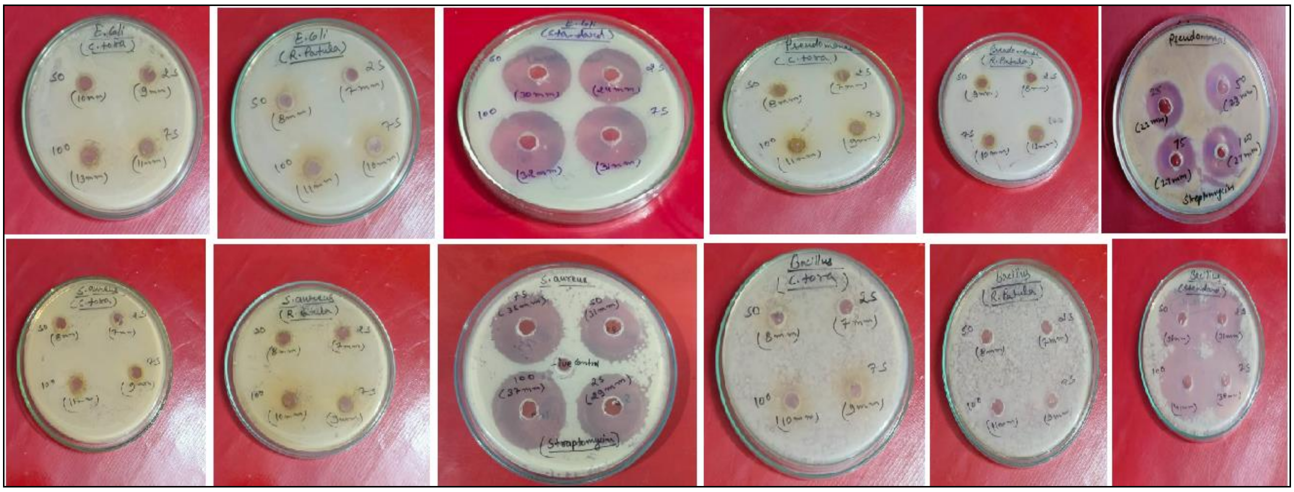

Fig 1: Antibacterial activity of the selected plant parts including standard antibiotic drug.

Discussion

The development of antimicrobial resistance to the existing antibiotics by disease causing microbes has fueled the research to hunt for newer antimicrobials. In this context, plants have been a valuable resource owing to their unparalleled ability to act as reservoir of bioactive primary as well as secondary metabolites, both of which are well renowned for usage under conditions of microbial attack, characterized by oxidative burst as well as inflammatory outburst **(**Elshafie *et al.,* 2023 [8]; Brusotti *et al.,* 2014**)** [4].

In this context, the results shown in current study further test the presence of primary metabolites, namely, total soluble sugars, proteins, and lipids in leaves of *C. tora* and *R. patula.* The results showcase presence of a good amount of all the primary metabolites in plants, with values of each of these parameters to be higher in *C. tora* in comparison to *R. patula*, However, primary metabolites are mainly involved in the overall growth and development of the plants and seldom play a role in plant defense system, as a consequence of which, primary metabolites are rarely reported to be antioxidant or antimicrobial in nature. However, primary metabolites still play a crucial role in plant development in a number of ways:

- Amino acids such as proline, aid in abiotic stress tolerance in plants by acting as osmolyte and controlling stomatal conductance as well as maintaining plant homeostasis *(*Khan *et al.,* 2020) [22].
- Several polyamines play a crucial role in providing tolerance to extreme temperatures in plants *(*Liu *et al.,* 2007) [28].
- Carbohydrates produced during photosynthesis provide much needed energy to the plants and also aid in maintenance of plant metabolism ***(***Patrick *et al.,* 2013**)** [34].
- Glycine betaine acts as osmoprotectant and provides drought tolerance ***(***Dikilitas *et al.,* 2020**)** [7].
- Plant lipids play a crucial role as active signaling molecules during both biotic as as well as abiotic stress in plants *(*Ali *et al.,* 2018) [1].

A number of other studies have also shown presence of primary metabolites in both of *C. tora* and *R. patula* ***(***Gaykhe *et al.,* 2017 [11]; Rani *et al.,* 2019 [37]; Lakshmi *et al.,* 2017**)** [25, 26]**.**

The results shown in the next section show presence of secondary metabolites, namely**,** total phenols, total flavonoids, free flavonoids, bound flavonoids, alkaloids, tannins and terpenoids) in leaves of both *C. tora* and *R. patula,* wherein, leaves of *C. tora* contained higher content of phenols whereas leaves of *R. patula* contained higher amount of total flavonoids, free flavonoids, bound flavonoids, alkaloids, tannins and terpenoids. Presence of secondary metabolites contributes to plant growth and development by fortifying the immune system of plants and protecting t from being attacked by nefarious pathogens, thereby strengthening the plant immune defense system. Furthermore, this very attribute of the secondary metabolites comes handy and has been commercially exploited for usage of these bioactive secondary metabolites for amelioration of a number of chronic and infectious conditions, characterized by marked oxidative unequlibrium as well as inflammatory outbursts.

This notion has bee put to test in the next section of the study, wherein, extracts from leaves of both these plants, namely, *C. tora* and *R. patula,* have been found to act as potent antioxidants, as evident from the results of DPPH assay, with higher antioxidant activity in case of *R. patula* in comparison to *C. tora.* This maybe attributed to higher concentration of total flavonoids, free flavonoids, bound flavonoids, alkaloids, tannins and terpenoids in *R. patula* in comparison to *C. tora.* All these secondary metabolites have been reported to act as potent antioxidants owing to their free radical scavenging activity reported in a number of previous studies (***(***Rehman *et al.,* 2017 [39] l Foti, 2007 [10]; Pietta, 2000 [35]; Gonzalez-Burgos *et al.,* 2012) [13].

Apart from antioxidant activity, the secondary metabolites also act as potent antimicrobials, thereby preventing the spread of nefarious bacterial infections by controlling the growth and multiplication of bacterial agents. This hypothesis was further put to test in the last section of the study, wherein, extracts from both leaves of both *C. tora* and *R. patula* were analyzed for the antibacterial efficacy against both gram positive as well as gram negative bacterial pathogens, namely, *Staphylococcus aureus, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa.* The results report the following findings:

- Higher antimicrobial activity of *C. tora* against *E. coli.*
- Equal antimicrobial activity of both *C. tora* and *R. patula* against *S.aureus.*
- Higher antimicrobial activity of *R. patula* against *B. subtilis* and *P. aeruginosa.*

The antimicrobial efficacy of both the plant extracts maybe attributed to the presence of bioactive secondary metabolites, namely, alkaloids, saponin, tannin, flavonoid, phenol, steroid, carbohydrate, glycosides, and terpenoids, all of which have been reported to exhibit potent antioxidant, anti-inflammatory and antibacterial activity (Koche *et al.,* 2016 [24]; Rex *et al.,* 2018 [40]; Gokhale *et al.,* 2015) [12].

The antibacterial action of both *C. tora* and *R. patula* maybe attributed to the presence of a number of bioactive compounds in these components, namely:

1. Tannins: Iron and nutrient deprivation leading to arrest of bacterial growth (Das *et al.,* 2019) [6]; Phosphorylation, enzyme, and protein synthesis inhibition in bacterial strains (Millones-Gómez *et al.,* 2020) [32].
2. Flavonoids: Bacterial cell wall disruption leading to bacterial killing (Umamaheswari *et al.,* 2020) [43]; Inactivation of microbial adhesion and protein transport (Millones-Gómez *et al.,* 2020) [32]
3. Terpenoids: Disruption of lipophilic compounds in bacterial membrane (Umamaheswari *et al.,* 2020) [43]
4. Saponins: Surface tension reduction leading to increase cell wall permeability and bacterial killing (Nugraha *et al.,* 2019) [33]
5. Phenolic Compounds: Bacterial membrane disruption and biofilm and virulence inhibition (Miklasińska-Majdanik *et al.,* 2022) [31]
6. Alkaloids: Inhibition of efflux pumps thus contributing to increased bacterial susceptibility to drugs (Jubair *et al.,* 2021) [18]

The results of this study showcasing potent antibacterial effect of *C. tora* and *R. patula* are in complete coherence with previous studies, where too, researchers have showcased the unparalled antimicrobial efficacy of both these plants and their extracts (Lee *et al.,* 2013 [27]; Das *et al.,* 2010 [5]; Sripriya 2014 [42]; Arulpandi *et al.,* 2011 [3]; Ramadevi *et al.,* 2016 [36]; Lakshmi *et al.,* 2017 [25, 26]; Seenivasan *et al.,* 2023) [41].

Conclusion

Considering the significant findings of the current study, it would not be wrong to label both *C. tora* and *R. patula* as a "wonder plants" owing to their astonishing antioxidant capability to alleviate pernicious disorders and ailments characterized by induction of oxidative burst. In addition to this, the current study also unveils the unparalleled efficacy of both the plant extracts to circumvent nefarious bacterial infections caused by both gram positive as well as gram-negative bacteria. However, the only limitation of the current study is it only shows results pertaining to antioxidant and antibacterial effect of the plant extracts and the molecular mechanism behind these effects is beyond the scope of this study.

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