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Original Research Article

Potentiation of Antifungal Activity of Coleus Aromaticus Leaves

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Abstract

Background: Many secondary metabolites found in plants, including tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., have been shown to exhibit antibacterial activities in vitro. Man has long been aware of herbal treatments. Traditional medical practitioners have detailed the therapeutic usefulness of numerous indigenous herbs for a variety of illnesses. More and more reports of medicinal plants' antimicrobial qualities are coming in from all over the world. By producing secondary metabolites with antibacterial characteristics, these plants offer another option for creating chemical fungicides that are both reasonably safe and reasonably priced. The Lamiaceae family member Coleus aromaticus is bitter, aromatic, digestively stimulating, stomachic, anathematic, deodorant, diuretic, and liver-tonic. ***Aim:*** The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some fungal strains. In the present study, the microbial activity of hydroalcoholic and methanolic extracts of leaves of *Coleus aromaticus* Linn. (an ethnomedicinal plant) was evaluated for potential antimicrobial activity against medically important fungal strains. ***Method:*** The antimicrobial activity was determined in the extracts using agar disc diffusion method. ***Result:*** The zone of inhibition for different strains of fungia i.e. *CA, CA, AN* were determined. The outcome of the investigation revealed that hydroalcoholic leaf extract of CA is more effective than methanolic extract as compare to standard due to presence of flavonoids. ***Conclusion:*** Both extracts (MeoH & HAE) showed effective antifungal activity, of which the HAE demonstrated the potent antifungal activity due to high flavonoid content.

Keywords: *Coleus aromaticus*, antifungal activity & fungia strain i.e. *CA, CA, AN*.

INTRODUCTION

New natural medicines are primarily derived from plants [1]. In an effort to pinpoint the chemical compounds found in medical plants that are responsible for treating human diseases, 74 percent of the 119 medications still taken from plants and still used around the world were found. Numerous uses, such as pharmaceuticals, alternative medicine, and natural therapies [2] have been built on the antibacterial properties of plant extracts. Plant pathogens including fungi, bacteria, nematodes, and viruses can harm plants or cause them to contract various diseases [3]. Fungi are the primary pathogens that damage plants, which is more significant. Worldwide, fungi diseases significantly reduce crop production in the agricultural sector. For instance, fungus like Fusarium spp. that grow on plants have the capacity to produce mycotoxins that can be extremely harmful to users. In the citrus industry, P. digitatum and P. expansum are responsible for orange rotting [3]. Mycotoxins include fumitoxins made by A. flavus and A. fumigatus as well as the aflatoxin B1 and B2 [4]. Antimycotics are used in agriculture to inhibit fungal development on plants and fruits, which is the first of its two key functions. Second, they can be utilised to stop or lessen the issue of post-harvest plant and fruit rotting [5]. The growth and presence of this fungus in food and animal feed endangers both human and animal health.

Predominant of Microbial Infection

Emerging infectious diseases in humans have become more common recently or are expected to do so in the near future. In the past three decades, more than 30 novel infectious agents have been found worldwide; 60% of these are zoonotic in nature. Given the intersection of current environmental, socioeconomic, and demographic factors, developing nations like India suffer disproportionately from the burden of infectious diseases [6]. One of the biggest threats to the effectiveness of the existing medical system is antibiotic resistance. Due to the advent of multidrug resistant bacteria and the fact that we can no longer be guaranteed that any antibiotic we choose will be effective, it has recently become increasingly dangerous. It is becoming increasingly obvious that antimicrobial resistance is simple to

produce but difficult to ignore. Any new agent raises serious concerns about resistance, which will only increase as new drug classes are developed. Because of the unrivalled abundance of chemical diversity, natural products, whether as pure compounds or as standardised extracts, offer endless prospects for new therapeutic leads in pharmaceutical studies. Therefore, to aid in this exceptional situation, natural goods need a strong and thorough assessment of their antibacterial qualities [7].

The herb Coleus aromaticus (CA), which is utilised for a number of purposes in many parts of the world, has several potential uses. The botanical genus Coleus, currently known as Plectranthus, and the herb Coleus aromaticus/amboinicus are both members of the Lamiaceae (Labiatae) family [8].

Coleus aromaticus

It is a big, succulent, fragrant perennial herb with thick, fleshy stems and leaves that is 30-90 cm tall. Pubescent herb with numerous branches and distinctively scented leaves. The plant is widely spread throughout India and grown in gardens. It is a traditional medicine that is used to cure a variety of ailments, including helminthiasis, colic, convulsions, epilepsy, renal and vesicular calculi, cough, chronic asthma, hiccough, bronchitis, and malarial fever [9]. Allelopathic potential, antibacterial and antimicrobial activity, insecticidal activity, components that scavenge free radicals and provide radioprotection from herb extracts, and most recently, the herb's culinary potential, are just a few of the herb's many potentials. The herb's flavour is mostly caused by carvacrol and thymol, while the phenolic components include chlorogenic acid, rosmarinic acid, etc. Both therapeutic and pharmacological uses of the herb as well as culinary preparations have been reported [10].

Experimental work

Procurement and authentication of Crude drugs:

The crude drug (leaves) was procured from locally from Shivpuri (M.P.) and authenticated. The drug was then allowed to dry in air and crushed in small pieces for extraction and extractive values.

Preparation of extracts

The powdered plant material (200gm) was extracted successively with redistilled, analytical grade petroleum ether (40-60^{O}C), chloroform, ethanol, methanol and water.

Qualitative Phytochemical analysis [11]

The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals.

Determination of Total Flavonoids Content

The total flavonoids content was estimated by $AlCl_3$ colorimetric method. The content of flavonoids was determined as quercetin equivalent. 10 mg/ml of plant extract in respective solvent (stock solution SS) was mixed with 2 ml $AlCl_3$ (2% w/v) in methanol and the solution was made up to 25ml with methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). 1ml of SS was made up to 25ml with methanolic solution of acetic acid (contrast solution CS). The absorbance of PS and CS was measured at 420nm after 30 minutes. The result expressed as % of total Flavonoids content [11].

%TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\ cm}$ x wt. of extract in gms

Evaluation of Antifungal Activity

The antifungal activity of hydroalcoholic leaves extract and standard antifungal amphotericin B was determined by disc diffusion methods and results obtained for Zone of inhibition was compared and reported [12].

Strain: *Candida albicans, Crytococcus neoformans* & *Aspergillus niger*.

Preparation of Disc

Disc of whatsmann filter paper of one quarter inch in diameter was prepared and the same was sterilized using autoclave.

Preparation of samples entrapped disc

The accurately weighed leaf extract of *C.aromaticus* was dissolved in methanol of different stock solutions (10, 20, 30, 40, 50 µg/ml**)** solutions were prepared. All the dilution prepared was applied to whatsmann filter paper disc using a micropipette. The disc was then dried and sterilized.

Preparation of culture plate

The sabouraund's agar and mueller Hinton agar media were prepared by dissolving media in 1000 ml of distilled water and sterilized by autoclave at 121°C for 1 hour. The media were cooled and poured in sterilized petri plate to solidified at room temperature.

Evaluation of Zone of inhibition

The re-cultured fungal strains were used for antifungal evaluation. The strains were streak on the Mueller Hinton media and the drug entrapped patches were placed. For negative control disc of distilled water and for positive control amphotericin B disc (10 µg) were used. The petri plates were kept in incubator for 24 hrs. After 24 hrs the petri-plates were checked for zone of inhibition. The zone of inhibition diameter was recorded with the help of zone reader scale. The zone of inhibition was calculated by subtracting diameter of sample or standard or control by diameter of disc. The more the zone of inhibition the more will be antifungal activity.

Statistical analysis

All the reading obtained were analyzed using one way analysis of variance i.e., ANOVA. Student t-test was used. The values are found to be statistically significant (*P<0.00, **P<0.01). All the values obtained are expressed as mean± standard error means (SEM).

RESULT & DISCUSSION

Plants have historically offered hope for fictional therapeutic molecules since plant herbal mixes have greatly benefited humans and its well-being. The popularity of using plant-based antimicrobial compounds as treatments for a variety of infectious illnesses has led to a high volume of searches for these compounds in plants. The anti-fungal activity of *C. aromaticus* leaf extract was assessed in the current experiment using methanolic and hydroalcoholic extracts.The preliminary phytochemical analysis of various leaf extract of *C.aromaticus* was tabulated in table 1. The result showed presence of flavonoids in methanolic extract and hydroalcoholic leaves extract of *C.aromaticus*. Further quantitative analysis of flavonoid was estimated by $AlCl_3$ colorimetric method. The result revealed that % TFC was found to be 1.48 & 2.42 in methanolic extract and hydroalcoholic leaves extract of *C.aromaticus*. respectively (table.2 & fig. 1).

The antifungal potential of both the extract was assessed in various fungal strains. Antifungal activity was estimated by disc diffusion method. The zone of inhibition for different strains of fungia i.e. *CA, CA, AN* were determined. The results were tabulated in table 3& fig. 2-3. The outcome of the investigation revealed that hydroalcoholic leaf extract of CA is more effective than methanolic extract as compare to standard due to presence of flavonoids. The antifungal potential may be endorsed due to the flavonoid and phenolic content present in the extracts.

Table 1: Phytochemical Screening of *Coleus aromaticus* leaves

Test	Pet.ether	Chloroform	Ethanolic	Methanolic	Hydro-alcoholic (70:30)
Carbohydrate					
Molish	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Benedict	(-)ve	(+)ve	(-)ve	(+)ve	(-)ve
Starch	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Hexose sugar	(-)ve	(-)ve	(-)ve	(+)ve	(-)ve
Tannin					
$FeCl_3$	(-)ve	(-)ve	(+)ve	(-)ve	(-)ve
Protein					
Biuret	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Xanthoprotein	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Amino acid					
Ninhydrin	(-)e	(-)ve	(-)ve	(-)ve	(-)ve
Alkaloids					
Dragnodroff	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Mayer	(-)e	(+)ve	(-)ve	(-)ve	(+)ve
Steroid					
Salkowski	(-)ve	(+)ve	(+)ve	(-)ve	(+)ve
Libermann –Bucher	(-)ve	(+)ve	(+)ve	(-)ve	(+)ve

Test	Pet.ether	Chloroform	Ethanolic	Methanolic	Hydro-alcoholic (70:30)
Flavonoids					
Shinoda	(-)ve	(-)ve	(-)ve	(+)ve	(+)ve
NaOH	(-)ve	(-)ve	(-)ve	(+)ve	(+)ve
Lead acetate	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Coumarin	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Glycosides					
Baljet	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Legal	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Killer-Killani	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

(+)ve = Present (-)ve Absent

Table 2: Total Flavonoids Content

S. No	Sample	%TFC
1.	Hydroalcholic extract of *C.aromaticus*	2.42
2.	Methanolic extract of *C.aromaticus*	1.48

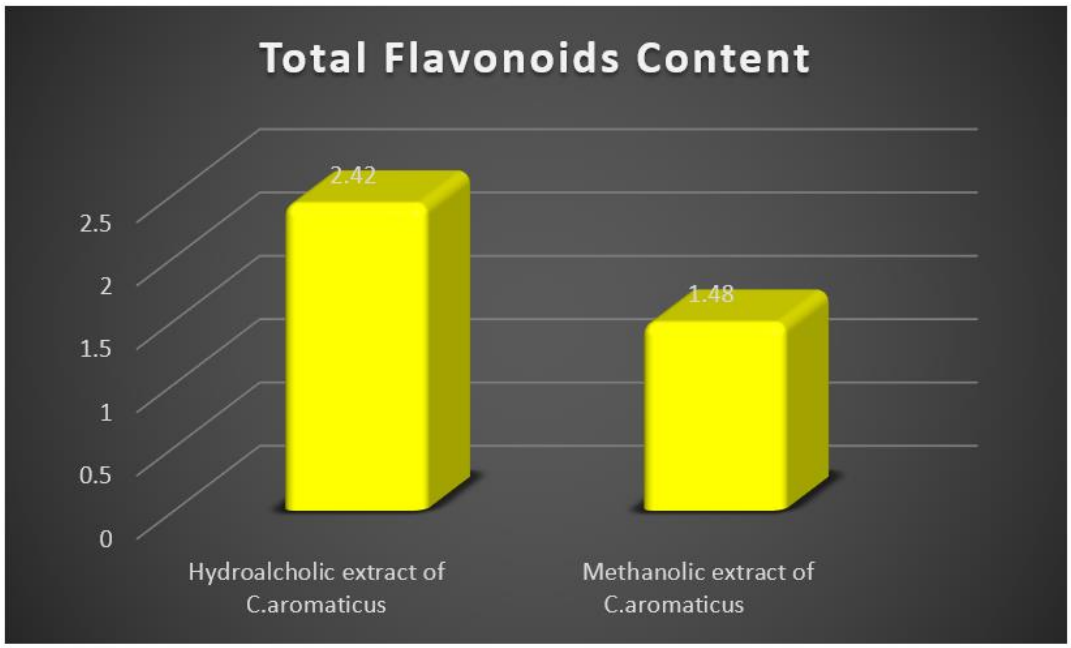


Fig. 1: % TFC

Table 3: Anti-fungal activity of leaf Extract of *C.aromaticus*

S/No.	Test/Extract	Zone of Inhibition (mm)		
		CA	CN	AF
1.	Negative Control	4.15±0.14	4.12±0.12	4.22±0.01
2.	Standard	20.29±0.12**	20.32±0.022**	20.42±0.18**
3.	Hydroalcoholic leaf extract	16.31±0.03**	19.09±0.10**	16.11±0.24**
4.	Methanolic leaf extract	13.22±0.10**	12.12±0.04**	12.50±0.01**

Note: All values are expressed as Mean (X) ±SEM, (n=3). One way ANOVA followed by student test, values are statistically significance *P<0.001, **P<0.01 when compared with control and standard.

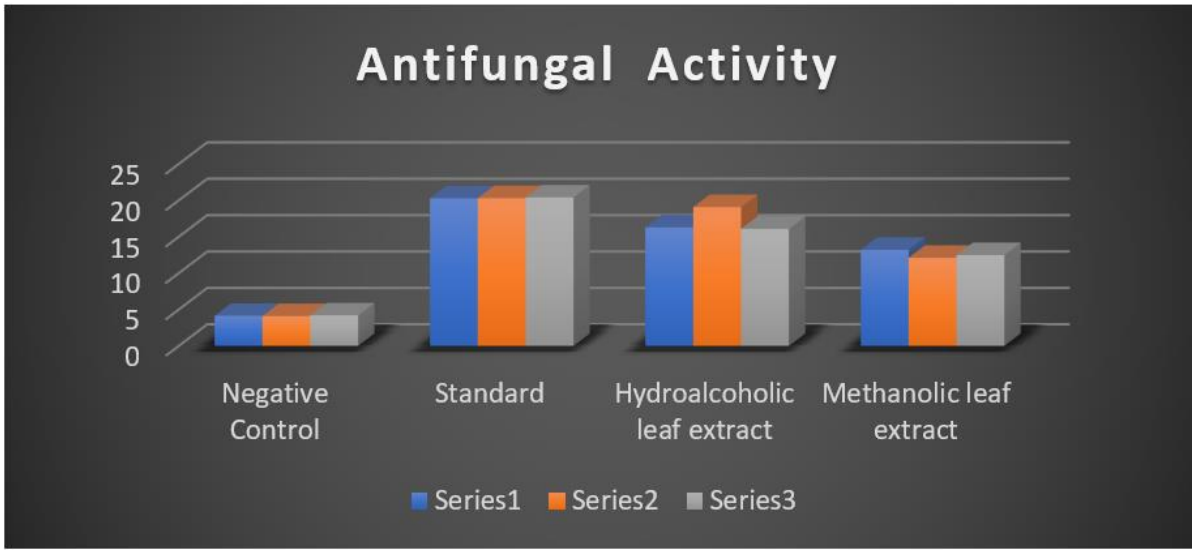


Fig. 2: Antifungal Activity
[Series 1 =CA, Series 2= CN, Series 3=AF]

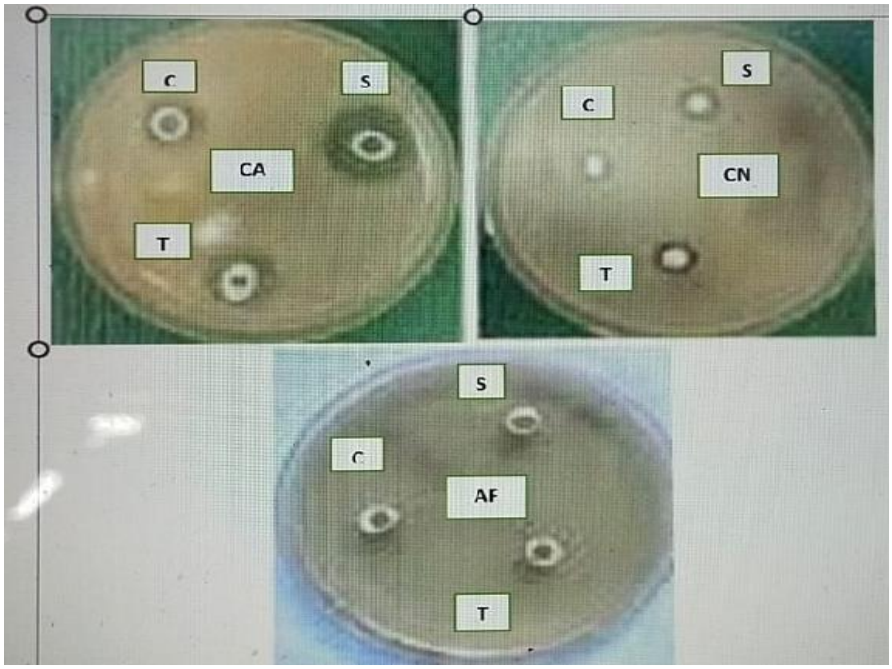
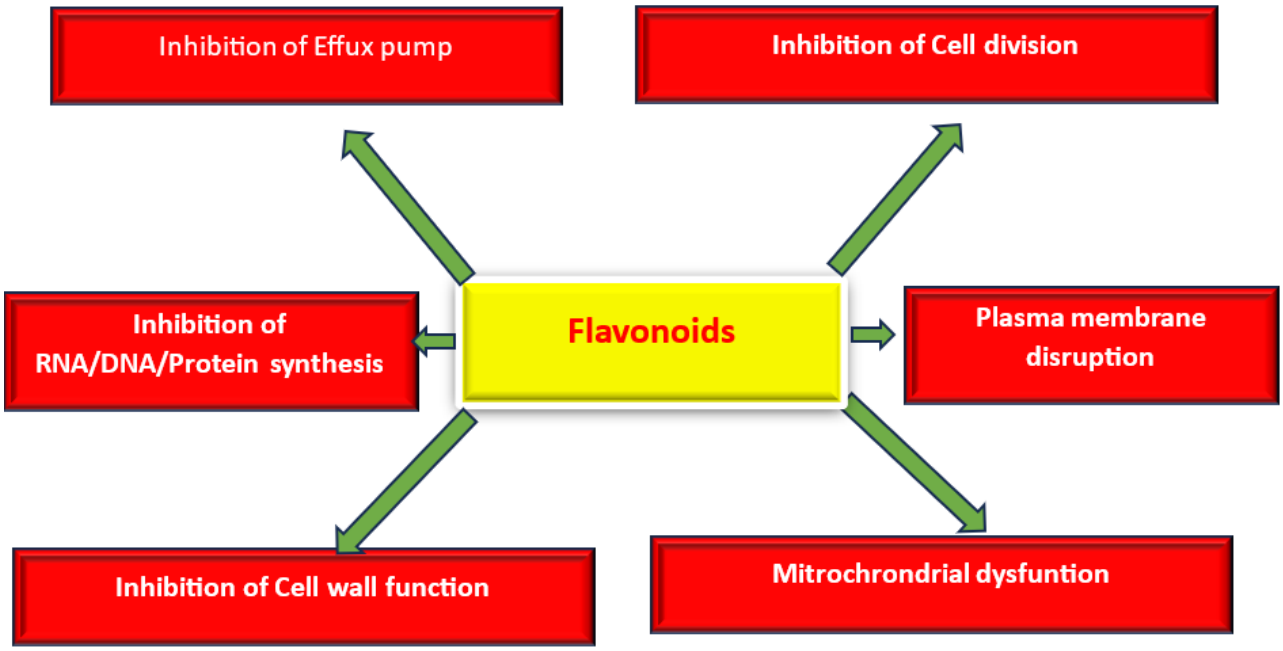


Fig. 3: Antifungal activity of leaf extract of *C.aromaticus*

CONCLUSION

The methanolic extract and hydroalcoholic extract of *C.aromaticus* leaves was taken in consideration for the current investigation. Leaves of the plant has been found to be a rich source of flavonoids & phenolic content. Moreover, the flavonoid and Phenolics present in the extract were analyses by chromatographic technique. The antifungal activity of both the extract was assessed in various fungal strains. Antifungal activity was estimated by disc diffusion method. Both extracts (MeoH & HAE) showed effective antifungal activity, of which the HAE demonstrated the potent antifungal activity due to high flavonoid content. As per outcome of current research work hydroalcoholic and methanolic leaves extract of *C.aromaticus* contained flavonoids more or less; therefore they have potentiate the antifungal acting due to one of the following possible mechanism:



REFERENCE

1. Hostettman, K. Strategy for the biological and chemical evaluation of plant extracts. IUPAC 1999.
2. Soni, H., Malik, J., Singhai, A. K., & Sharma, S. (2013). Antimicrobial and antiinflammatory activity of the hydrogels containing rutin delivery. *Asian Journal of Chemistry*, *25*(15), 8371.
3. Montesinos, E. (2003). Development, registration and commercialization of microbial pesticides for plant protection. *International microbiology*, *6*, 245-252.
4. Mahlo, S. M., McGaw, L. J., & Eloff, J. N. (2010). Antifungal activity of leaf extracts from South African trees against plant pathogens. *Crop Protection*, *29*(12), 1529-1533.
5. Mahlo, S. M., Chauke, H. R., McGaw, L., & Eloff, J. (2016). Antioxidant and antifungal activity of selected medicinal plant extracts against phytopathogenic fungi. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(4), 216-222.
6. Amor, G., Caputo, L., La Storia, A., De Feo, V., Mauriello, G., & Fechtali, T. (2019). Chemical composition and antimicrobial activity of Artemisia herba-alba and Origanum majorana essential oils from Morocco. *Molecules*, *24*(22), 4021.
7. Gowdhami, M., B.L. Sarkar, & P.M. Ayyasamy. (2014). Screening of phytochemicals antibacterial activity of Annona squamos aextracts. *Int. J. Pharm. Sci. Invent*.3: 30–39.
8. Himesh, S., Singhai, A. K., Malik, J. K., & Sarvesh, S. (2012). Evaluation of Leaves of Aqueous Extract of Coleus Aromaticus and Methanolic Extract of Annona Squamosa Extracts on Cell Viability. *American J. Pharm Tech Res*, *2*(4), 936-944.
9. Himesh, S., Ak, S., & Sarvesh, S. (2012). Quantitative estimation of DNA isolated from leaves and stem of Coleus aromaticus. *Int J Pharm*, *2*(1), 84-89.
10. Soni, H., Nayak, G., Patel, S. S., Mishra, K., Singhai, A. K., Swarnkar, P., & Pathak, A. K. (2011). Synergistic effect of polyherbal suspension of Punica granatum and Coleus aromaticus in evaluation of wound healing activity. *J Herbal Med Toxicol*, *5*(1), 111-115.
11. Himesh, S., Singhai, A. K., & Sarvesh, S. (2011). Quantitative estimation of DNA isolated from various parts of Annona squamosa. *IRJP*, *2*, 12.
12. Bhalodia, N. R., & Shukla, V. J. (2011). Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *Journal of advanced pharmaceutical technology & research*, *2*(2), 104-109.