**Journal of Neonatal Surgery**
***ISSN(Online): 2226-0439***
***Vol. 14, Issue 10s (2025)***
*https://www.jneonatalsurg.com*

OPEN ACCESS

# Phytochemical Characterization and Anti-Acne Potential of Cassia fistula and Plumeria obtusa Extracts Against Cutibacterium acnes

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Cite this paper as: Pallavi Tripathi, Nikunj Agarwal, Sanjiv Kumar Chaudhri, Daisy Arora, Bharat Khurana, (2025) Phytochemical Characterization and Anti-Acne Potential of Cassia fistula and Plumeria obtusa Extracts Against Cutibacterium acnes. *Journal of Neonatal Surgery*, 14 (10s), 82-94.

**ABSTRACT**

The *Cassia fistula* and *Plumeria obtusa* extracts were evaluated for their anti-acne efficacy against the key pathogen in acne vulgaris, *Cutibacterium acnes*, with respect to their phytochemical composition. This research explores plant derived, antimicrobial and antioxidant properties with the increasing antibiotic resistance and toxic effects of conventional treatments. Flavonoids, alkaloids, terpenoids, polyphenols, steroids, glycosides were chemical compounds identified in phytochemical analysis of the extracts already known to be bioactive. The bioactive constituent's resveratrol, cinnamic acid and plumericin along with derivatives from andrographolide received validation through Nano Liquid Chromatography coupled Tandem Mass Spectrometry (Nano LC/MS/MS) analysis. Higher concentration of resveratrol was seen in *Plumeria obtusa*, which have higher potential for pharmacological activity seen as compared to the *Cassia fistula*. *Plumeria obtusa* showed better free radical neutralization (IC50 = 1050.91 µg/mL) than by the DPPH radical scavenging assay. Both extracts have a biocompatibility demonstrated by their cytotoxicity evaluation using Vero cells and then used in dermatological application. Microdilution assays showed that *C. acnes*was significantly inhibited with *Plumeria obtusa* having superior activity over *Cassia fistula*, which were attributed at higher polyphenol and flavonoid content only. These findings lend support for therapeutic use of these plant extracts in acne. *Plumeria obtusa*, showing better antimicrobial and antioxidant activity than other species, becomes a promising candidate of phyto therapeutic formulation. Additional investigations utilizing *in vivo* evaluations and development of formulation is then necessary to achieve clinical applicability and to confirm effectively.*Cassia fistula* and *Plumeria obtusa* extracts were evaluatedwhich has higher potential. These findings support the therapeutic use of these plant extracts in acne.

***Keywords:*** *Acne vulgaris, Phytochemicals, Antibacterial activity, Antioxidant properties, Cassia fistula, Plumeria obtusa*

## 1. INTRODUCTION

As a physical barrier against harmful agents, the skin is the protective tissue covering the whole human body. It almost entirely blocks the body from surrounding microorganisms. Other than that, damage to the skin allows its protection to be compromised, leading to increased risk of bacterial infections [1-3]. Acne is a common dermatological condition which occurs in 70 – 90 % of adolescents and a large number of adults, particularly women [4,5]. The multiple causal factors behind this condition arise from *Cutibacterium acnes* (formerly Propionibacterium acnes) which is a gram-positive anaerobic bacterium.*C. acnes* is part of the normal skin microbiota but overgrowth or dysregulation of *C. acnes* can lead to inflammation, which may result, or contribute to, the development of acne lesions [6, 7]. Acne does not stop at physical signs such as comedones, cysts: it also causes scarring, pigmentation and psychological problems such as low self-esteem and anxiety [8-10]. Therapy difficulties become more challenging because Acnes strains exist thus requiring additional therapeutic approaches. In this study, the prevalence of acne and its association with C. acnes are explored as a study of pathogenesis and potential treatment for the disease [11, 12]. Common therapies for conventional acne include topical

retinoids, antibiotics, or benzoyl peroxide, which reduce inflammation and bacterial growth, but can cause drying, irritation and resistance [8, 13, 14]. Oral antibiotics and isotretinoin systemic options are more severe cases but offer risks to include gastrointestinal issues, photosensitivity and liver toxicity. Weight alterations and blood clot formation represent several adverse side effects linked to sebum regulation by oral contraceptives and anti-androgen medications [15, 16].In addition to physical therapies (such as chemical peels and laser treatments) there may also be redness, irritation, and they can be costly [17, 18]. The multitude of bioactive molecules produced by plants makes them a good source of natural medicines, including treatments for microbial infections.Plant extracts and products have been used as functional ingredients in foods, cosmetics and detergents as well as in traditional medicine for centuries [19–21]. Notably, among the antimicrobial active plant extracts and essential oils, there exist those against pathogens associated with infection such as*Cutibacteriumacnes*. Particularly effective against Gram positive, as well as Gram negative bacteria, fungi and viruses [22, 23]. As essential oils have been proved to have bactericidal and bacteriostatic properties, they can prove to be beneficial in controlling those skin pathogens like *C. acnes*. Antimicrobial activity of these plants is mostly due to oxygenated terpenoids like alcohols and phenolic terpenes, some of the hydrocarbons may also contribute to their activity. However, many of these components are synergistic and can extend their efficacy against microbial strains, including acne causative strains [24]. Herbal medicinal products are economically and medicinally important throughout the world (particularly their use in dermatological applications). Despite their wide acceptance within the population the quality safety and efficacy of herbal products continues to raise considerable concerns [25-27]. There are major plant-based formulations available for alternative therapies for the skin conditions associated with acne and *C. acnes* is the main contributor [28]. Accession to natural origin drugs (NODs) consumed by 80 percent of the world's population dictates that progress is made through research in the development, evaluation, and standardization of herbal formulations aimed for *C. acnes* [29]. Rich phytochemical composition and therapeutic properties of *Cassia fistula* and *Plumeria obtusa* make them promising natural remedies for treatment of acne [30–32]. Compared with other studied trees, *Cassia fistula* is rich in bioactive flavonoids, alkaloids, anthraquinones, and tannins, of which these compounds impart probable antimicrobial, anti-inflammatory, antitumor, antihistamine, and antioxidant activity agents. These attributes make it effective against acne causing bacteria (*Cutibacterium acnes*), decrease inflammation and oxidative stress from acne lesions. Just like *Plumeria obtusa* which has broad spectrum antimicrobial properties and affects pathogenic bacteria such as skin infections. In addition to its anti-inflammatory and skin healing properties, *Cassia fistula* also has additional potential for acne symptom reduction and skin regeneration [39]. Recent researches have verified its antimicrobial and anti-inflammatory performance at low concentration in topical form, and P. obtusa has also significant antioxidant activity and *C. acnes* inhibitory capability [33]. In this study we did a comprehensive examination of phytochemicals and antimicrobial activity of plant extracts against acne bacteria (*Cutibacterium acnes*). Methods involving certain tests on terpenoids, steroids, alkaloids, flavonoids, glycosides, polyphenols, monosaccharides and proteins with the Salkowski, Liebermann-Burchard, and Biuret test were applied for the phytochemical screening. In addition, anti-bacterial activity of the extracts at different concentrations was also assessed using microdilution methods. Polyphenols were quantified with advanced tests such as Folin-Ciocalteuand Ferric Chloride assays; Scientists employed Benedict's and Barfoed's tests for determining reducing sugar content.The final findings revealed that *Plumeria obtusa* contains a higher concentration of resveratrol compared to *Cassia fistula*. This supports the antimicrobial potential and bioactive properties of these plant extracts, highlighting their promise for developing natural therapies to manage acne.

## 2. MATERIALS AND METHODS

### *2.1 Materials*

The phytochemical tests in this study were carried out using sulfuric acid, chloroform, acetic anhydride and hydrochloric acid (1%) which were all purchased from Fischer scientific. For alkaloid detection, Dragendorff's reagent, Mayer's reagent and picric acid solution were taken from Sisco research laboratories; and sodium hydroxide and glacial acetic acid were used for flavonoid and glycoside tests respectively and were obtained from Rankem. Ferric chloride, Folin-Ciocalteu reagent, and 7.5% sodium carbonate were employed for polyphenol evaluation and was bought from Alpha Chemika. Benedict's reagent and Barfoed's reagent were utilized for monosaccharide identification and was purchased from HiMediaLaboratories. Biuret reagent and ninhydrin reagent were used for protein testing was bought from Merck India. Antibacterial tests used Mueller-Hinton Broth (MHB) along with dimethyl sulfoxide (DMSO) for bacterial culture while extract dilution material came from Nice Chemicals and Thermo Fisher Scientific India. The analysis used chemicals from an analytical grade.

### *2.2 Methods*

#### *2.2.1 Phytochemical Screening*

Phytochemical screening plays a crucial role in identifying the bioactive compounds present in plant extract.

##### *2.2.1.1 Test for Terpenoid*

The presence of terpenoids was assessed using the Salkowski and Liebermann-Burchard tests.

#### *2.2.1.1.1 Salkowski test*

The solution containing 100 mg of crude extract received two measured 2 mL volumes of chloroform in a test tube before a drop-wise addition of 2 mL of concentrated sulfuric acid began at the tube's sides. The interface development of a reddish-brown colourconfirm the presence of terpenoids in the study [40].

#### *2.2.1.1.2 Liebermann-Burchard test*

An initial addition of chloroform followed by acetic anhydride occurred to the 0 mg extract. After chilling the sample rapidly it received a 2 mL addition of concentrated sulfuric acid. The interface showed a brown ring as a sign of steroid development and triterpenoid presence generated a deep red reaction [41].

### *2.2.1.2 Test for Alkaloids*

15 mg extract was agitated with 6 mL 1% hydrochloric acid in a water bath for 5 minutes, filtered and then tested for alkaloids. Three parts of the filtrate were subjected to specific tests.

#### *2.2.1.2.1 Dragendorff's test*

The reagent Dragendorff's reagent (1 mL) was added, which gave an orange red precipitate indicating alkaloids.

#### *2.2.1.2.2 Mayer's test*

In this test, Mayer's reagent was added giving a cream-colored precipitate.

#### *2.2.1.2.3 Hager's test*

The extract was made alkaline and the presence of alkaloids was confirmed by the formation of a yellow precipitate made by acidifying the extract with picric acid solution [42].

### *2.2.1.3 Test for Flavonoids*

Thus, Alkaline Reagent Test was performed to test Flavonoids. The test solution turned colorless when treated with a diluted acid, showing that the added sodium hydroxide gave a strong yellow color that was the characteristic of flavonoids [43].

### *2.2.1.4 Test for Glycosides*

Glycosides were detected by the Keller-Kiliani test. A mixture of 2 mL of extract, glacial acetic acid, and a few drops of ferric chloride was layered with concentrated sulfuric acid. The formation of a blue color at the interface indicated the presence of deoxy sugars and glycosides [44].

## *2.2.2 Antibacterial Activity*

### *2.2.2.1 Preparation of Bacterial Culture*

Bacterial strains were cultured in Mueller-Hinton Broth (MHB) and incubated at 37°C with shaking (180 rpm) until they reached the log phase ($OD_{600}$ ~ 0.5–0.6). The cultures were diluted to achieve a final concentration of ~$5 \times 10^5$ CFU/mL for use in microdilution assays (Clinical and Laboratory Standards Institute[45].

### *2.2.2.2 Preparation of Test Compounds*

Stock solutions of the plant extracts were prepared in dimethyl sulfoxide (DMSO). Serial two-fold dilutions were performed in MHB to achieve a range of concentrations (100–200 µg/mL). The prepared solutions were used for antibacterial testing [46].

### *2.2.2.3 Microdilution Assay*

In a sterile 96-well plate, 100 µL of MHB was added to each well, followed by 50 µL of the diluted extract and 50 µL of bacterial suspension. The plates were incubated at 37°C for 24 hours under aseptic conditions. Antibacterial activity was measured by assessing bacterial growth inhibition using a microplate reader at $OD_{600}$ nm. A lower optical density indicated effective bacterial inhibition [47].

## *2.2.3 Polyphenols evaluation*

### *2.2.3.1 Ferric Chloride Test*

The test solution was mixed with 1% ferric chloride. The appearance of green, blue, or purple coloration indicated the presence of polyphenols [48].

### *2.2.3.2 Folin-Ciocalteu Test*

The extract (0.5 mL) was combined with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was incubated at room temperature for 30 minutes, and absorbance was measured at 760 nm. A blue color indicated the presence of polyphenols, which were quantified using a gallic acid standard curve[49].

### *2.2.4 Monosaccharides*

#### *2.2.4.1 Benedict's Test*

One millilitre of Benedict's reagent was added to the extract (1 mL) and heated in a boiling water bath. The appearance of green, yellow, or brick-red precipitate confirmed the presence of reducing sugars[50].

#### *2.2.4.2 Barfoed's Test*

The extract (1 mL) was mixed with Barfoed's reagent (1 mL) and heated. The rapid formation of a red precipitate within 2 minutes indicated monosaccharides [51].

### *2.2.5 Proteins*

#### *2.2.5.1 Biuret Test*

The extract (1 mL) was treated with 2 mL of Biuret reagent. A violet color formation indicated the presence of proteins [52].

#### *2.2.5.2 Ninhydrin Test*

One milliliter of ninhydrin reagent was added to the extract and heated in a boiling water bath for 5 minutes. A purple or blue color confirmed the presence of amino acids or proteins [53].

## 3. RESULTS

### *3.1 Chemical Tests of Cassia fistula and Plumeria obtusa*

The chemical analysis of *Cassia fistula* extract was conducted using standard phytochemical tests to determine the presence of bioactive compounds.The results confirmed the presence of terpenoids, polyphenols, alkaloids, flavonoids, and proteins in the *Cassia fistula* extract. However, monosaccharides, some alkaloid-specific reactions, and glycosides tested negative

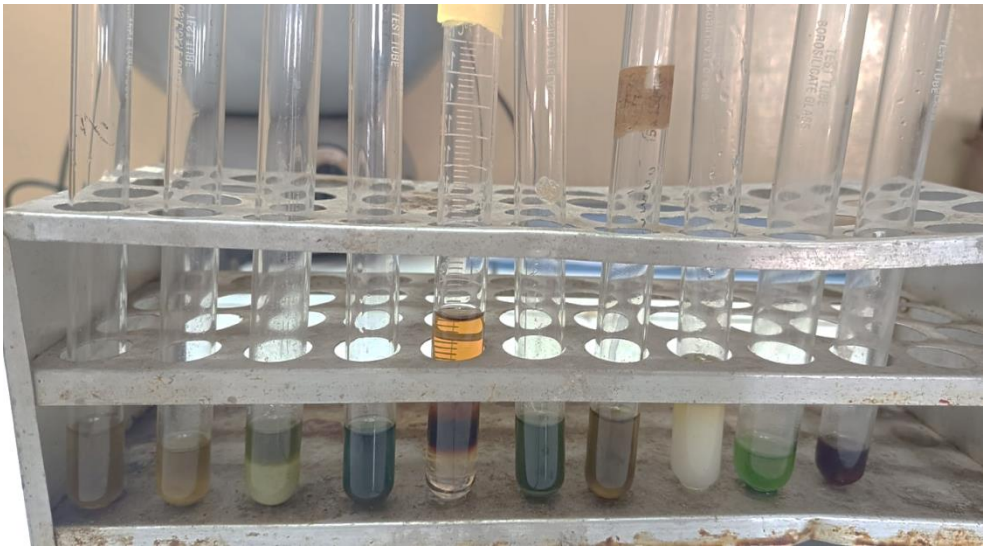

**Figure 1: Figure displays test tubes with results from various phytochemical tests. The observed color changes indicate the presence or absence of compounds like alkaloids, terpenoids, flavonoids, polyphenols, glycosides, and proteins.**

### *3.2 Phytochemical Analysis of Plumeria obtusa*

The phytochemical analysis of *Plumeria obtusa* extract demonstrated the presence of several bioactive compounds, highlighting its therapeutic potential. The extract tested positive for alkaloids, triterpenoids, steroids, flavonoids, saponins, polyphenols, and glycosides, as shown in Table 1.

**Table 1: Phytochemical test analysis of *Plumeria obtusa***

| S No. | Phytochemical Test | Presence |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Triterpenoids | + |

| | | |
|---|---|---|
| 3 | Steroids | + |
| 4 | Flavonoids | + |
| 5 | Saponins | + |
| 6 | Polyphenols | + |
| 7 | Glycosides | + |

.

### *3.3 Phytochemical Analysis of Cassia fistula*

The phytochemical analysis of *Cassia fistula* extract revealed the presence of several bioactive compounds, as determined by specific chemical tests. The extract tested positive for terpenoids, polyphenols, alkaloids, flavonoids, and proteins, while monosaccharides and glycosides were not detected (Table 2). The findings of these results put emphasis on the major phytochemical profile of Cassia fistula whose pharmacological potential depends on this.

**Table 2: Phytochemical test analysis of *Cassia istula***

| **S No.** | **Category** | **Phytochemical test** | **Results** |
|---|---|---|---|
| 1 | Terpenoids | Salkowski test<br>Liebermann Burchard test | +<br>+ |
| 2 | Polyphenols | Phenolic reagent test | + |
| 3 | Monosaccharides | Barford test | - |
| 4 | Alkaloids | Dragendorff's test<br>Mayer's test<br>Hager's test<br>Wagner's test | +<br>+<br>-<br>- |
| 5 | Flavonoids | Alkaline reagent test | + |
| 6 | Glycosides | Keller kiliani test | - |
| 7 | Proteins | Molisch test | + |

### *3.4 Cassia fistula analysis by Nano Liquid Chromatography coupled with Tandem Mass Spectroscopy.*

*Cassia fistula* extract was subjected to Nano Liquid Chromatography coupled with Tandem Mass Spectroscopy analysis revealing diverse and rich phytochemical composition, apart from being a source of other bioactive compounds. Alkaloids, flavonoids and polyphenols (which are all known for having important therapeutic properties in the body) are some of the compounds that are identified noteworthily. These findings confirm the definition of *Cassia fistula* as a valuable source of natural drugs. The extract has also been run on liquid chromatography the chromatogram of which is shown in Figure 2 and the attributes of the compounds identified are listed in Table 3. Resveratrol in the *Cassia fistula* extract has been identified by Nano LC/MS/MS to be at a peak height of 125,584.

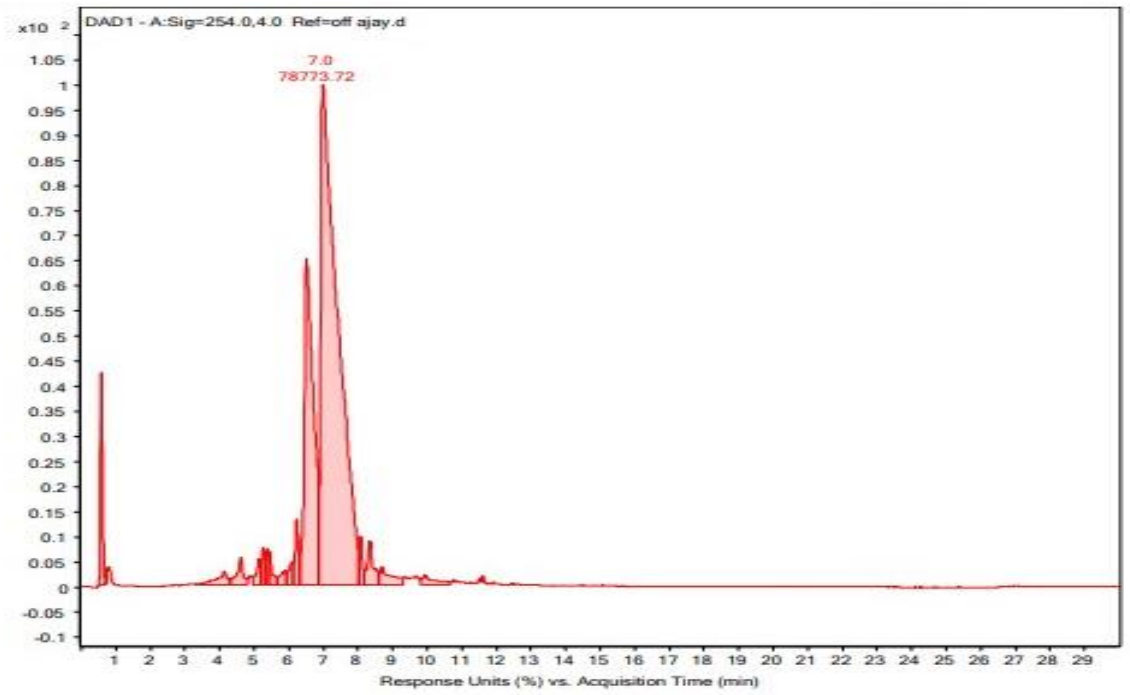


**Figure 2-LC/MS chromatogram of peaks of the *Casia fistula extracts***

**Table 3- Represent the compounds present in the *Cassia fistula***

| | | | | | | Compound Identification | hyd |
|---|---|---|---|---|---|---|---|
| **Formula** | **m/z** | **Mass** | **RT** | **Height** | **Score** | **Name** | **Diff (Tgt, ppm)** |
| C21 H21 N O6 | 384.1441 | 383.1368 | 6.22 | 970100 | 98.95 | 1S,9R-Hydrastine | -0.25 |
| C20 H18 N O4 | 337.1254 | 336.1262 | 6.76 | 975206 | 11.44 | Berberine | 7.65 |
| **C20 H21 N O4** | **262.1379** | **228.240** | **9.15** | **125584** | **77.02** | **Resveratrol** | **5.33** |
| C21 H25 N O4 | 356.1851 | 355.1778 | 6.07 | 45516 | 98.21 | Dl-tetrahydropalmatine | -1.43 |
| C11 H13 N O3 | 208.0963 | 207.0888 | 3.69 | 36812 | 79.93 | Hydrastinine | -3.66 |
| C21 H21 N O6 | 384.1441 | 383.1368 | 6.22 | 970100 | 98.95 | 1S,9R-Hydrastine | -0.25 |
| C20 H21 N O4 | 362.1379 | 339.1489 | 9.15 | 125584 | 77.02 | DL-Canadine | 5.33 |
| C21 H25 N O4 | 356.1851 | 355.1778 | 6.07 | 45946 | 98.21 | Dl-tetrahydropalmatine | -1.43 |

### *3.5 Nano Liquid Chromatography coupled with Tandem Mass Spectroscopy for Plumeria obtusa*

The analysis of *Plumeria obtusa* extract using Nano Liquid Chromatography coupled with Tandem Mass Spectroscopy for identified multiple bioactive compounds, showcasing its diverse phytochemical profile (Figure 3). The identified compounds include alkaloids, flavonoids, and polyphenols, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. Significant compounds such as cinnamic acid, resveratrol and plumericin were detected, along with various andrographidine derivatives. These findings emphasize the therapeutic potential of *Plumeria obtusa* in pharmacological applications. The identified compounds are summarized in Table 4.

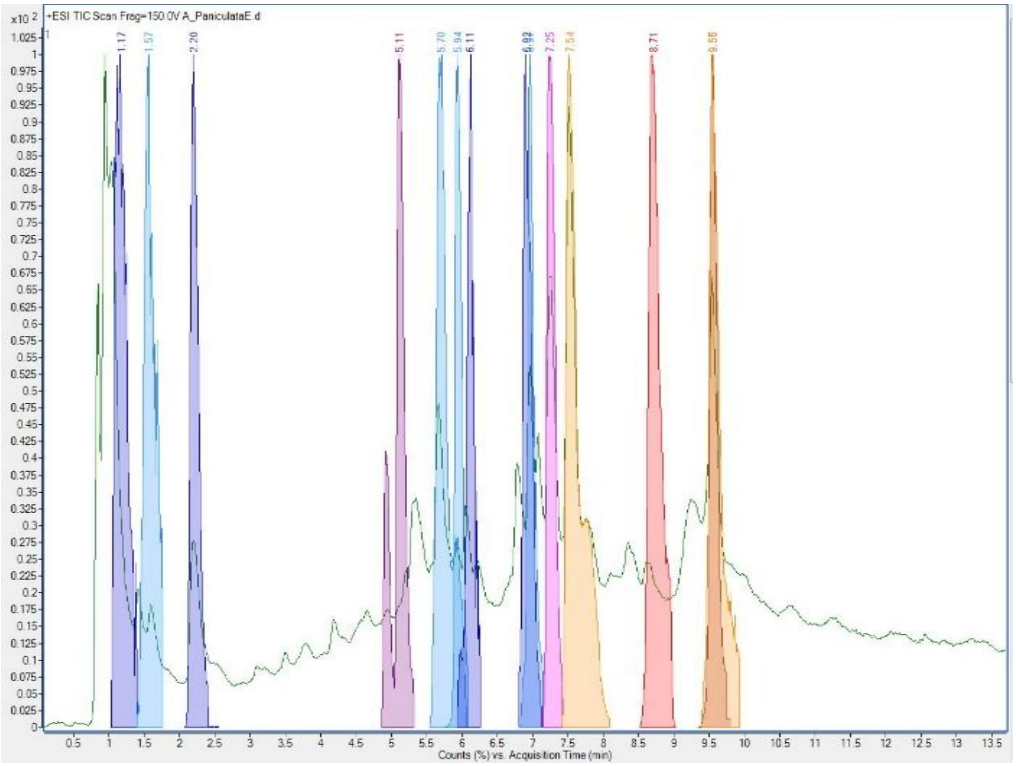


**Figure 3-LC/MS chromatogram of peaks of the *Plumeria obtusa***

**Table 4- Represent the compounds present in the *Plumeria obtusa***

| **General** | | | | | | | | **Compound Identification** |
|---|---|---|---|---|---|---|---|---|
| **Formula** | **m/z** | **Mass** | **RT** | **Width** | **Avg Mass** | **Score** | **Height** | **Name** |
| C9 H8 O2 | 149.0592 | 148.0519 | 1.17 | 0.23 | 148.0519 | 45.76 | 7125 | Cinnamic acid |
| C23 H24 O12 | 515.1158 | 492.1265 | 1.57 | 0.21 | 492.3693 | 73.33 | 5246 | Androfraphidine B |
| C16 H18 O9 | 293.335 | 292.334 | 2.2 | 0.14 | 292.334 | 86.83 | 1290582 | Isoplumericin |
| C23 H24 O11 | 499.1199 | 476.1308 | 5.11 | 0.23 | 476.4946 | 86.84 | 40262 | Andrographidine D |
| C19 H28 O5 | 359.1817 | 336.1924 | 5.7 | 0.28 | 336.3825 | 78.68 | 42988 | Isoandrographolide |
| C20 H32 O5 | 448.720 | 426.720 | 5.94 | 0.11 | 426.720 | 83.75 | 1545742 | Lupanol |
| C23 H24 O10 | 483.125 | 460.1356 | 6.11 | 0.16 | 460.3789 | 68.44 | 3798 | Andrographidine C |
| C20 H30 O5 | 373.1967 | 350.2074 | 6.92 | 0.15 | 350.3362 | 66.12 | 1186257 | Andrographolide |
| C24 H26 O11 | 513.137 | 490.1481 | 6.97 | 0.11 | 490.441 | 64.81 | 5953 | Andrographidine E |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| C20 H28 O4 | 333.2046 | 332.1972 | 7.25 | 0.13 | 332.3385 | 73.23 | 1178849 | deoxy-didehydroandrographolide |
| **C19 H28 O7** | **286.1925** | **228.2490** | **8.71** | **0.22** | **228.2490** | **74.49** | **3693438** | **Resveratrol** |
| C20 H24 O2 | 292.184 | 296.1766 | 9.55 | 0.14 | 292.3046 | 73.49 | 1640322 | Plumericin |
| C23 H26 O10 | 485.142 | 462.1529 | 9.56 | 0.27 | 462.3176 | 79.83 | 41567 | Andrographidine A |

The Nano LC/MS/MS analysis demonstrated that the *Plumeria obtusa* extract exhibited a resveratrol peak height of 3,693,438, indicating significantly higher abundance compared to the *Cassia fistula* extract, which showed a resveratrol peak height of 125,584.The retention time (RT) 8.71 minutes appears to be where Resveratrol is the main active compound of *Plumeria obtusa* extract. These findings support the idea that *Plumeria obtusa* is a richer source of Resveratrol, a compound known for its antioxidant and anti-inflammatory properties, as another remarkable phytochemical profile in both extracts. This comparative analysis highlights the possible therapeutics of *Plumeria obtusa* and his additional contribution to Resveratrol based pharmacological effects.

### *3.6 DPPH Inhibition Assay*

Antioxidant activity of the extracts was evaluated by performing the DPPH (2,2 diphenyl–1 picrylhydrazyl) inhibition assay. The method is based on the capacity of antioxidants to act as scavengers of the Purple stable DPPH free radical and thus produce colour from Purple to Yellow which can be quantified Spectrophotometrically. Fig. 4 shows that *Plumeria obtuse* possessed the better antioxidant effect than *Cassia fistula* with IC50 of 1050.91.

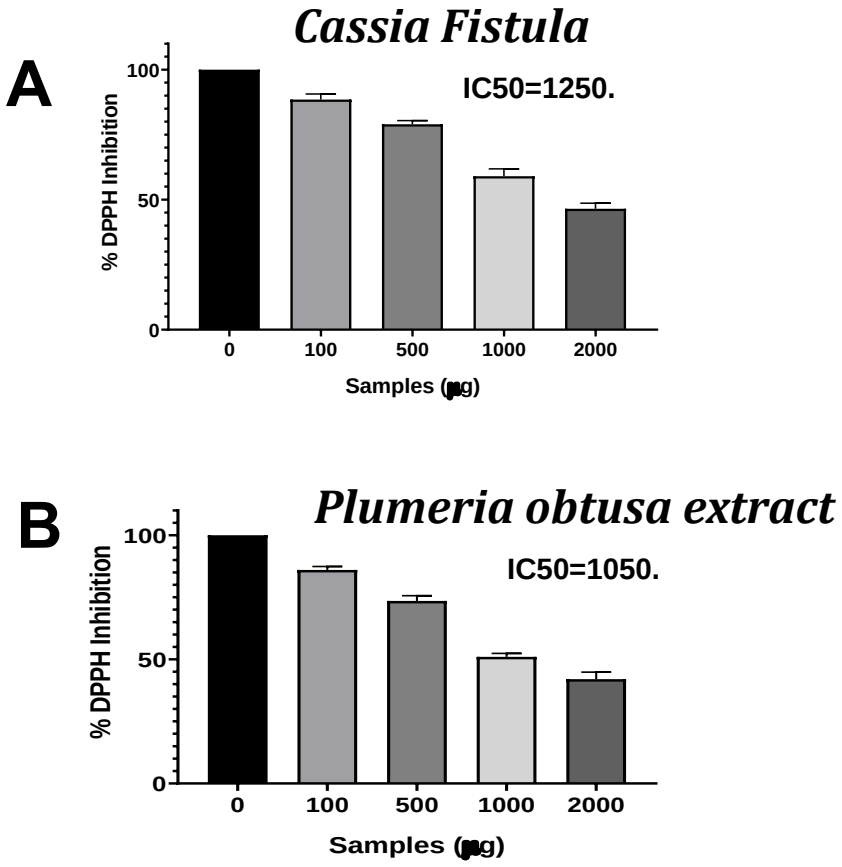


**Figure 4:(A) DPPH assay of *Cassia fistula*on different concentrations(B)DPPH assay of *Plumeria obtuse* on different concentrations**

### *3.7 Cell Viability assay*

We treated Vero cells with 100, 500, 1000, and 2000 µg for 24 hours to gauge the extract's potential cytotoxicity. The MTT test was used to assess cytotoxicity, and the results can be shown in Figure 3. Cellular viability did not show any significant changes after exposure. The extracts were shown to be non-cytotoxic to normal human cells, according to the cell viability experiment (Figure 5).

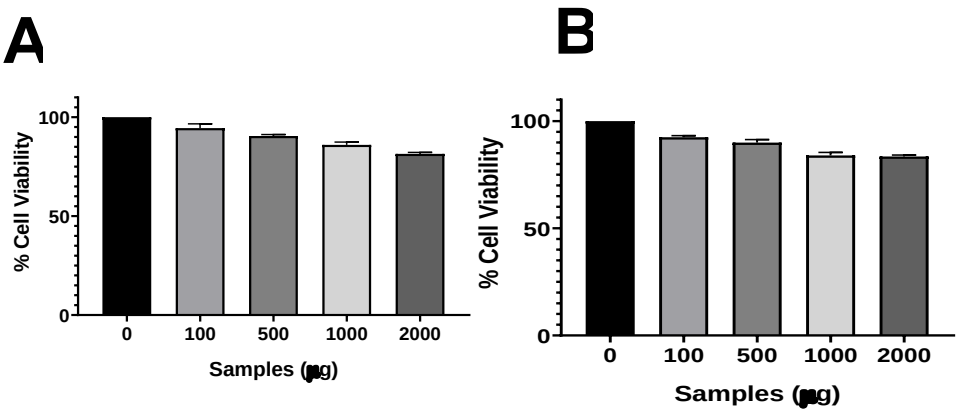


**Figure 5- Cell viability assay of (A) Cassia fistula and (B) Plumeria obtuse on different concentrations on Vero cells.**

### *3.8 Anti-Acne activity*

In this study, the antibacterial activity of *Cassia fistula* and *Plumeria obtusa* extracts was evaluated against *Cutibacterium acnes* at varying concentrations (500, 1000, 1500, and 2000 µg). The effectiveness of the extracts was determined based on $IC_{50}$ values. The results indicate that *Plumeria obtusa* exhibited superior antibacterial activity with an $IC_{50}$ value of 1050.34 µg, whereas Cassia fistula demonstrated slightly lower efficacy, with an $IC_{50}$ value of 1145.78 µg. These findings suggest that Plumeria obtusa could serve as a potential natural alternative for antibacterial applications against acne-causing bacteria (Figure 6).

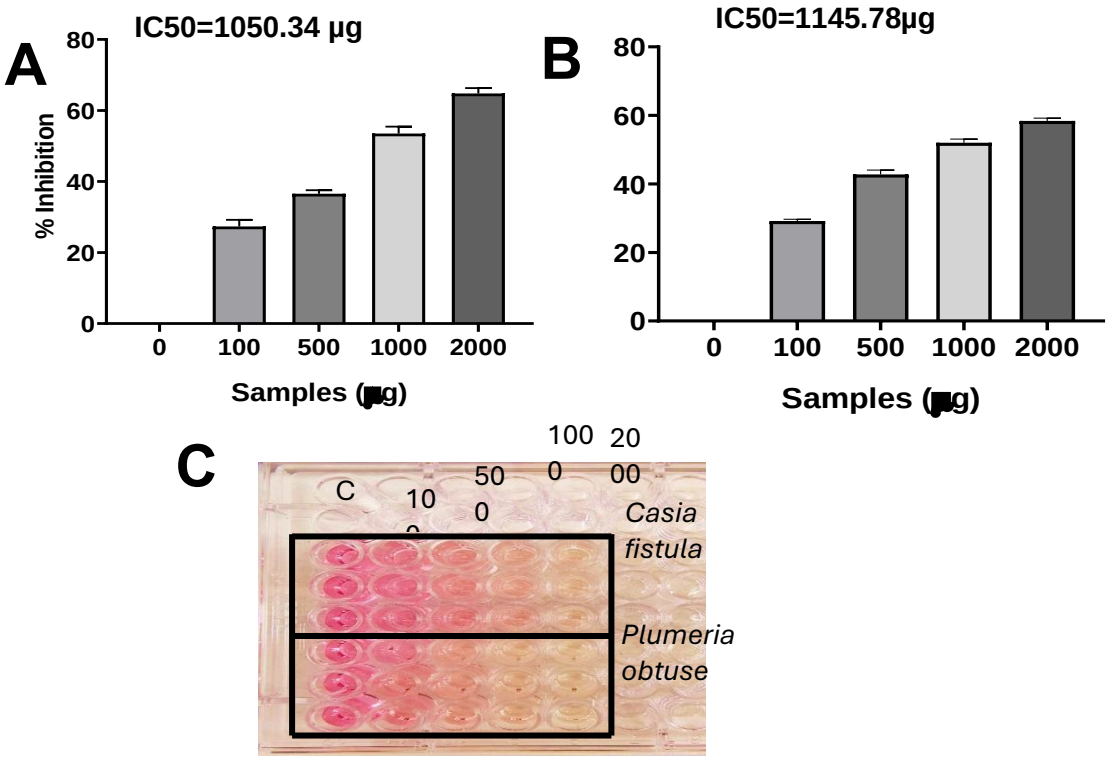


**Figure 6: Antibacterial activity ofassay of (A) *Plumeria obtusa* (B) *Cassia fistula*on different concentrations of*Cutibacterium acnes*, and (C) 96 trans-well plate assay of *Plumeria obtusa* and *Cassia fistula* against *Cutibacterium acnes*.**

## 4. DISCUSSION

This study was done to investigate the phytochemical composition, antioxidant potential and antibacterial efficacy of *Cassia fistula* and *Plumeria obtusa* extracts against *Cutibacteriumacnes.* A phytochemical analysis showed the existence of bioactive compounds such as flavonoids, alkaloids, terpenoids, polyphenols, steroids and glycosides, which are proven to possess antimicrobial, anti-inflammatory and antioxidant properties. The secondary metabolites of both plant extracts were diverse. However, the alkaloids, polyphenolic substances, terpenoids, and flavonoids in *Cassia fistula* were dominant while those in *Plumeria obtusa* included high contents of alkaloids, steroids, triterpenoids, and flavonoids along with polyphenolic substances. These compounds indicate that both extracts could have wide pharmacological applications. The Nano LC/MS/MS analysis showed that the concentration of resveratrol in *Plumeria obtusa* was much higher than that in *Cassia fistula*.The antioxidant activity of both extracts was determined by evaluation in the DPPH radical scavenging assay. Results showed that *Plumeria obtusa* had a higher antioxidant potential with lower IC50 value (1050.91 µg/mL) than *Cassia fistula*. The antioxidant activity in the treatment of acne is important as oxidative stress plays a role in inflammation and aggravates the development of acne pathogenesis. Both extracts showed the non-cytotoxic nature using cell viability assay on Vero cells. However, we found that neither *Cassia fistula* nor *Plumeria obtusa* significantly affected cell viability, as demonstrated by the MTT assay, so they are safe for potential therapeutic applications. The antibacterial activity by both plant extracts against *C. acnes* was very significant, and *Plumeria obtusa* demonstrated its slightly more effective than Cassia fistula. From the IC50 values of the antibacterial activity, it showed that the *Plumeria obtusa* had a better antibacterial activity (IC50 = 1050.34 µg/mL) than *Cassia fistula* (IC50 = 1145.78 µg/mL). These extracts are probably antibacterial due to their flavonoids, alkaloids and polyphenols, also known to destroy bacterial cell walls and impede biofilm formation. It could be the fact that *Plumeria obtusa* poses antibacterial performance better due to the presence of resveratrol which possess antimicrobial and anti-inflammatory properties.

This study demonstrates a promising plant-based alternative from which the plant extracts could be used with fewer adverse effects and the possibility of synergistic benefits to manage acne. These extracts show the antioxidant, anti-inflammatory, and antibacterial properties and thus can be incorporated in newly developed topical formulations for the treatment of acne. The study indicates the therapeutic potential of these plant extracts and more *in vivo* and clinical trials are required to prove efficacy and safety in humans. Furthermore, formulation studies on these extracts in topical creams or gels need to be done to also assess stabilities, absorption, and bioavailability of the extract.

In future research, interactions between this agent and other natural or synthetic anti-acne agents should also be investigated. Over 5 different anti-acne activity of cassia fistula and *Plumeria obtusa* extract were studied in this work and showed that all these extracts are phyto chemically rich, antioxidant present and antibacterium against *cutibacterium acnes*. Resveratrol in *Plumeria obtusa* conferred stronger antibacterial and antioxidant effects.According to the findings, thisplant-based extract could be good safe, effective and natural alternatives to conventional acne treatments, with less risk of resistance to antibiotics and lesser side effect.

## 5. CONCLUSION

In this study, the phytochemical characterization, as well as the anti-acne potential of *Cassia fistula* and *Plumeria obtusa* extracts on *Cutibacterium acnes*, were investigated systematically. Despite addressing this problem, acne vulgaris continues to haunt the skin suffering antibiotic resistant and other undesirable effects that usually accompany treatments used for this condition. With the increasing attention to phytotherapeutics worldwide, the present work was undertaken to elucidate antimicrobial, antioxidant, and cytotoxic properties of these plant extracts and emphasize such extracts as life option natural alternatives against acne. The secondary metabolites present in the plants like flavonoids, alkaloids, terpenoids, polyphenols, steroids and glycosides are known to have antimicrobial, anti-inflammatory and antioxidant properties were verified by phytochemical analysis. Resveratrol, cinnamic acid, plumericin, and andrographidine derivatives were identified by Nano LC/MS/MS and *Plumeria obtusa* showed greater particle of resveratrol that may have stronger biological activity. It was found that the antioxidant potential of *Plumeria obtusa* was higher than other plants as it had IC50 value of 1050.91µg/mL in DPPH radical scavenging assay. Cell viability assay demonstrated that both extracts were non cytotoxic and thus indicated their suitability for dermatological application. *Plumeria obtusa* had the superior efficacy against C. acnes (IC50 = 1050.34 µg/mL) than *Cassia fistula* (IC50 = 1145.78 µg/mL) as a result of its higher flavonoid and polyphenol content.

The study suggests that *Plumeria obtusa*is a more potent natural anti-acne agent due to its stronger antibacterial and antioxidant effects. Further *in vivo* studies and formulation research are recommended to optimize their clinical application and ensure therapeutic efficacy.

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