Phytochemical and Antibacterial Assessments of Leaves of *Carthamus tinctorius* L. in Chhattisgarh

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Abstract

Carthamus tinctorius L. is a wild edible plant that is widely consumed in several regions of Chhattisgarh as well as the neighboring states. In this study, we investigated the phytochemical composition and antibacterial activity of the leaves of *C. tinctorius* L. collected from different locations. The phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, carbohydrates, flavonoids, tannins and phenolic compounds. It was found that the leaves of *Carthamus tinctorius* L. are rich in flavonoids, phenolics, and tannins. The concentration of Phenolics was found to be highest (173.945 +-2.214 µg GAE/ mg Plant Extract), followed by Tannins (49.486 +- 2.06) µg GAE/mg Plant Extract and Flavonoids (11.552 +- 0.335 µg Quercetin Equivalent /mg Plant Extract). The antibacterial assay showed that the extract exhibited significant inhibitory activity against all tested bacterial strains i.e, *Staphylococcus aureus*, , *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The extract showed the highest activity against *Pseudomonas aeruginosa*, with a zone of inhibition 29.6 ± 1.818 mm and the lowest activity against *Klebsiella pneumoniae*, with a zone of inhibition of 9.4±1.818. Overall, our findings suggest that the leaves of *C. tinctorius* L. from Chhattisgarh contain a range of bioactive compounds with significant antibacterial activity against pathogenic bacteria.

Keywords: *Carthamus tinctorius* L., Phytochemicals, Antibacterial, pharmacological, Chhattisgarh.

1. Introduction

Carthamus tinctorius L., commonly known as Safflower or false saffron, is a thistle-like herbaceous crop that is self-compatible and annual. It is diploid, with a chromosome count of 2n=24. Safflower thrives in hot and dry climates and is believed to have been domesticated more than 4,000 years ago in the Fertile Crescent region (Weiss, 1983). It belongs to the Asteraceae family within the order of Asterales, which encompasses a vast number of genera and species, totaling approximately 22,750 genera and over 1,620 species (Knowles, Ashri, 1995).

Carthamus species are thought to have originated from Southern Asia and have been cultivated in various regions such as China, India, Iran, and Egypt since prehistoric times. During the middle Ages, safflower cultivation spread to Italy, France, and Spain. It was later introduced into the

United States in 1925 from the Mediterranean region. In Iran, *C. tinctorius* is commonly referred to as "Golrang" (Zargari, 1988). The plant is grown for the red/orange pigment found in its flower petals, which is used to color rice, bread, and dye cloth (Zhao,Zhao,Tu, 2009) . However, with the rise of synthetic aniline dyes in the 1800s, safflower cultivation shifted towards oilseed production (Weiss, 1983).

C. tinctorius is described as a bushy, herbaceous annual plant with multiple branches. The branches are categorized as primary, secondary, and tertiary, each terminating in a globular structure called capitulum. The stems and branches are covered in spiny leaves (Madaan et al, 2011).

Safflower is primarily grown as an oilseed crop under dry land conditions. It produces white, shiny, and smooth seeds called achenes. These fruits may or may not have pappus (tufts of hair) and are four-sided with a thick pericarp. Each branch of safflower produces a globular flower capitulum, which is surrounded by tightly attached bracts. Safflower possesses a taproot system that can extend to 2-3 meters in soils with adequate depth. The deep root system enables safflower to extract water and nutrients from deeper soil layers compared to other crops, making it suitable for rain-fed cropping systems (Weiss, 1983).

This plant is primarily cultivated for its seeds, which are used for edible oil production and as birdseed (Kumar, Kumari, 2011). The flowers of safflower were historically utilized for food coloring, flavoring, and dye production. In certain regions, such as Turkey and Iran, safflower has gained importance as a crop due to the high nutritional value and rich content of its edible oil (Zargari, 1988). The oil derived from safflower seeds contains a significant amount of polyunsaturated fatty acid linoleic acid (70%) and monounsaturated oleic acid (10%), with small quantities of stearic acid.Apart from these, *Carthamus tinctorius* is abundant in an extensive range of secondary metabolites that have been shown to have antibacterial effects, such as tannins, terpenoids, saponins, alkaloids, and flavonoids (Punjanon, Arpornsuwan, Klinkusoom, 2004). Owing to the presence of these biologically active compounds, this plant has been used in traditional medicine for treating various ailments, including fever, asthma, cough, and liver disorders. The plant is also used in the cosmetic industry for making hair oil, skin lotion, and soap. (Ekor, M., 2013). These compounds have been shown to have various significant activities, including antioxidant, anti-inflammatory, antibacterial, and anticancer activities.(Afsana et al.2021) Therefore, the identification and characterization of phytochemicals present in medicinal plants are essential for the development of new drugs. Interestingly, plant-based biocides are non-

phytotoxic, comparatively harmless and systemic and readily biodegradable compared to chemotherapeutics (Nega, A., 2014).

In this study, we aim at evaluating the phytochemical composition of the leaves of *Carthamus tinctorius* L. collected from the different locations of Chhattisgarh. This evaluation of *Carthamus tinctorius* L. can provide valuable insights into the chemical constituents and potential therapeutic properties of this plant.

2. Methodology

The leaves of *Carthamus tinctorius* L. were collected from various locations of Chhattisgarh. The plant was identified and authenticated by a taxonomist at the Department of Botany, Government Science College, Chhattisgarh.

2.1 Preparation of Plant Extract:

The leaves were washed thoroughly with distilled water and shade-dried at room temperature. Using a grinder, the dried leaves were crushed to a fine powder. The powdered leaves (50g) were extracted using 95% ethanol (500 ml) by Soxhlet extraction method. The extract was concentrated using a rotary evaporator and then lyophilized to obtain a dry powder.

2.2 Qualitative determination of phytochemicals

The method outlined by Sofowora (1993) and Evans (1998) was used for phytochemical analysis to check the plants for the presence of alkaloids, flavonoids, and carbohydrates.

Test for Alkaloids: The 10% tannic acid solution was combined with a few drops of the manager's reagent, Drangendorff's reagent, Wanger's reagent, or Hanger's reagent. Precipitate in at least three or all of the aforementioned reagents showed the presence of alkaloids.

Test for Carbohydrates: Two tubes containing 2 ml of each water extract, added a few drops of Molisch's reagent added to them. Then a little amount of conc. H_2SO_4 was added, and a lower layer was allowed to form. The presence of carbohydrates is shown by a purple ring at the liquid-liquid interface. Each mixture was then mixed, left to stand for 2 minutes, and then diluted with 5ml of water. Additionally, the presence of carbohydrates was indicated by a purple precipitate.

Test for Proteins: Add a few drops of the Ninhydrine solution gently to 1 ml of plant extract, then allow it to come to a boil. When it does, the appearance of blue colour indicates that the plant extract contains amino acids.

Test for Tannins: A small amount of the water extract was mixed in a 1:4 ratio with distilled water, and a few drops of a 10% ferric chloride solution were also added. A blue or green colour indicated tannins were present.

Test for Flavonoids: Four pieces of magnesium fillings was added in ethanolic extract followed by few drops of concentrated hydrochloric acid. A pink or red colour was indicated the presence of flavonoid (Selvakumar *et.al 2012*)

2.3 Quantitative determination of phytochemicals

1. Estimation of Phenolics

The Folin-C reagent, a complex mixture of heteropolyphosphotungstatemolybdate, was used to first extract the phenols in water, which they then combined with to create a blue-colored complex while being in the presence of sodium carbonate. The number of reactive phenolic compounds in the sample has a direct relationship to the intensity of the blue color. By measuring the sample solution's absorbance at 765 nm and contrasting it with a calibration curve using Gallic acid as a reference, the amount of phenolic in the sample was determined. (Kala, C. P., 2006)

A Folin-Ciocalteu reagent was used to assess the phenolic contents in plant extract using a UV-spectrophotometric technique. In a volumetric flask with a capacity of 25 ml, the assay mixture consists of 1 ml of an extract and 9 ml of water. The flask was filled with one ml of folin-ciocalteu reagent and thoroughly shaken. The liquid volume was increased to 25ml and 10ml of 7% sodium carbonate was added after 5 minutes. The same procedure was used to generate standard solutions of gallic acid (20, 40, 40, 60, 80, and 100 g/ml), and after 90 minutes of incubation, absorbance at 550 nm was measured with the help of a UV spectrophotometer. The extract's total phenol content was given as g of GAE/mg.

2. Estimation of Tannin Content

The tannin content was calculated using the Folin-Ciocalteu technique. In this procedure, a 10 ml volumetric flask containing 0.1 ml of the sample extract and 7.5 ml of distilled water was used. After adding 0.5 ml of the Folin-Ciocalteu phenol reagent, the flask was thoroughly shaken and

allow remain at room temperature. Gallic acid standard solutions were made at 20, 40, 60, 80, and 100 µg/ml concentrations. Using a UV/VIS spectrophotometer, the absorbance of the test and standard solutions was measured against prepared blank solutions at 725 nm. (Ribarova, F.*et.al* 2005)

3. Estimation of Total Flavanoid Content

The aluminium chloride colorimetric assay was used to measure the total flavonoid content of the leaf extract. For the experiment, 1 ml of plant extract was combined with 4 ml of distilled water, then 0.3 ml of 5% NaNO2 was added. 0.3 ml of 10% AlCl3 was added after 5 minutes. A 10 ml volumetric flask was then filled to the desired level with 2 ml of 1M NaOH. Following the same process, a series of standard medicines, Quercetin, were made utilising concentrations of 20, 40, 60, 80, and 100 g/ml. Using a Labtronics spectrophotometer model LT-2201, the absorbance of the standard and test solutions was assessed at 510 nm after a 30-minute incubation period. (Pandey, A. K. *et.al* 2023)

2.4 Screening of Antibacterial Activity

By using the Kirby-Bauer (Bauer et al., 1966) disc diffusion method, plant extract was tested for its inhibitory efficacy against the test bacteria (Doughari, J.H. 2006). For inoculating diffusion plates, the inoculum size taken was 1-2 x 108 CFU/ml of bacteria, equivalent to the McFarland 0.5 turbidity standard (Yisa, J. 2009). After the standard disc and test disc have been placed on the plate, the plate was incubated at 37°C for 24 hours. Each test was performed three times with controls. Ampicillin, Amphotericin and Steptomycin were used as control antibiotic for the gram negative and gram positive bacteria respectively. The plates were then incubated for 48 hours at 37±1ºC in an incubator and the petri plates were observed for the inhibition of bacterial growth by the extracts. And the results were calculated by measuring the diameter of zone of inhibition of extract around the well by the help of a measurement scale in millimeters.

3. Results and Discussion

3.1 Phytochemical Screening:

The phytochemical screening of the leaf extract of *Carthamus tinctorius L.* revealed the presence of various bioactive compounds, including Carbohydrate, Proteins, Alkaloids, Flavonoids, Tannins and Phenols. The results are presented in Table 1.

Table1: Qualitative phytochemical analysis of *Carthamus tinctorius L*. Ethanolic Extract

S.No.	Plant Constituents *Carthamus tinctorius L*	Test	Ethyl acetate extract
1	Carbohydrate	Molisch test	Present
2	Protein	Ninhydrin test	Present
3	Alkaloids	Dragendoffr's test	Absent
4	Flavonoids	Shinoda test	Present
5	Tannins	Lead acetate test	Present
6	Phenols	Ferric chloride test	Present

Numerous beneficial phytochemicals, like alkaloids, flavonoids, phenolics, tannins, proteins and carbohydrates, were found in the ethanolic extract of *Carthamus tinctorius* leaf. Flavonoids have antibacterial, antidiarrheal, and antioxidative activity, while alkaloids are known to have antihelmintic, antibacterial, and antidiarrheal properties. In addition to having antibacterial, antidiarrheal, and antihelmintic properties, phenols and tannins have also exhibited antioxidant properties.

Estimation of Phenolic Compound

Using the Folin-Ciocalteu reagent method, the phenolic content was estimated. The plant extract contained a significant amount of phenolic compounds. Total phenolic compounds were expressed in terms of Gallic Acid Equivalent (GAE). The value obtained for the concentration of

total phenolic compounds is expressed as µg GAE/mg of plant extract. The total phenolic content examined in the plant extract is 173.945 µg GAE/mg of plant extract. Phenolic compounds were calculated by using the formula (y = 0.00299x - 0.0183) (Shown in Figure 1).

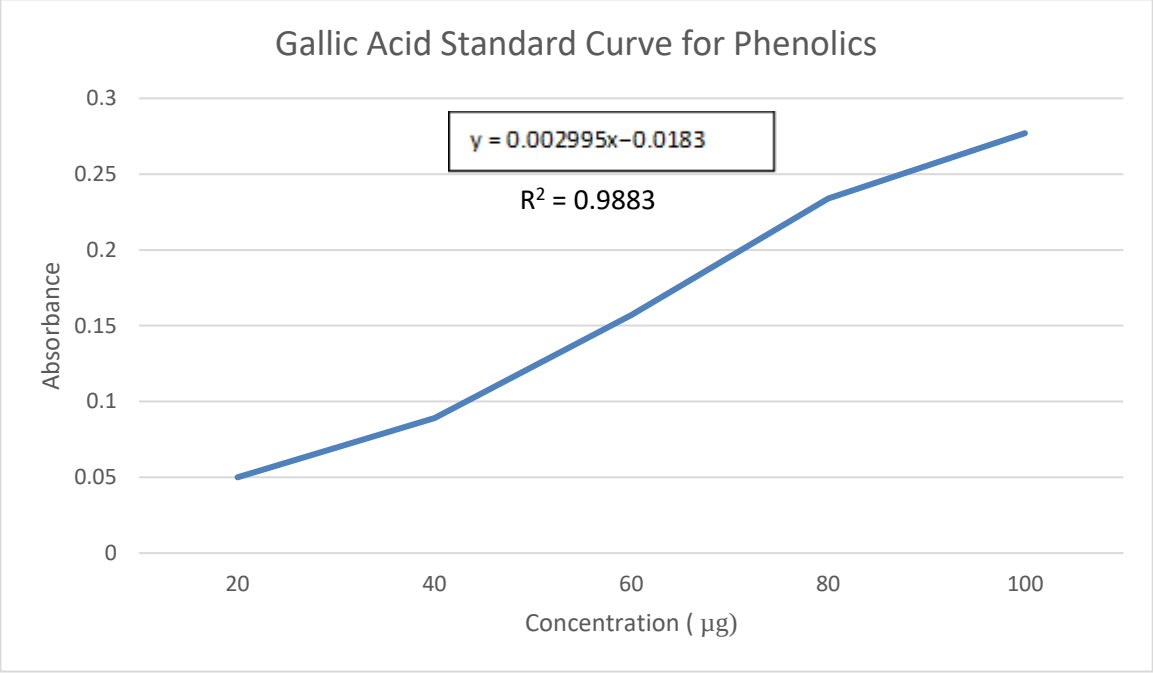

Fig.1 Gallic acid standard curve for phenolic content

Estimation of Tannins

The estimation of the tannins was also done using Folin-Ciocalteu reagent. The total Tannin content was also measured in Gallic Acid Equivalents. The concentration of the total tannin component obtained is defined as µg GAE/mg of plant extract. The total tannin content of the plant extract was measured as 49.486 µg GAE/mg of plant extract. Tannin compounds were calculated by using the formula (y=0.00624x-0.1162) (shown in Figure 2).

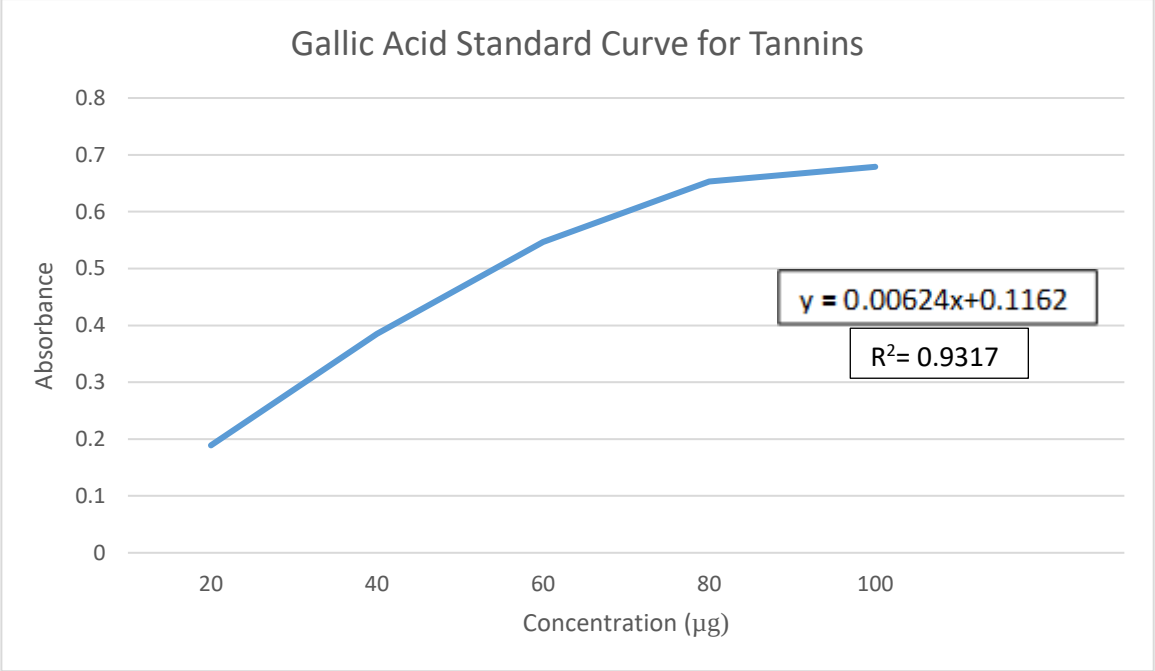

Fig.2 Gallic acid standard curve for Tannin content

Estimation of Flavonoids

The estimation of Flavonoid compounds was done by Aluminum Chloride Colorimetric assay. A high level of flavonoid content was found in the plant extracts. The total flavonoid compounds were measured in Quercetin Equivalents. The concentration of the total flavonoids obtained is reported as µg quercetin/mg of plant extract. The total flavonoid content of the plant extract was measured as 11.552 µg quercetin equivalent /mg of plant extract. Flavonoid compounds were calculated by using the formula (y=0.00895+0.01566) (shown in Figure 3).

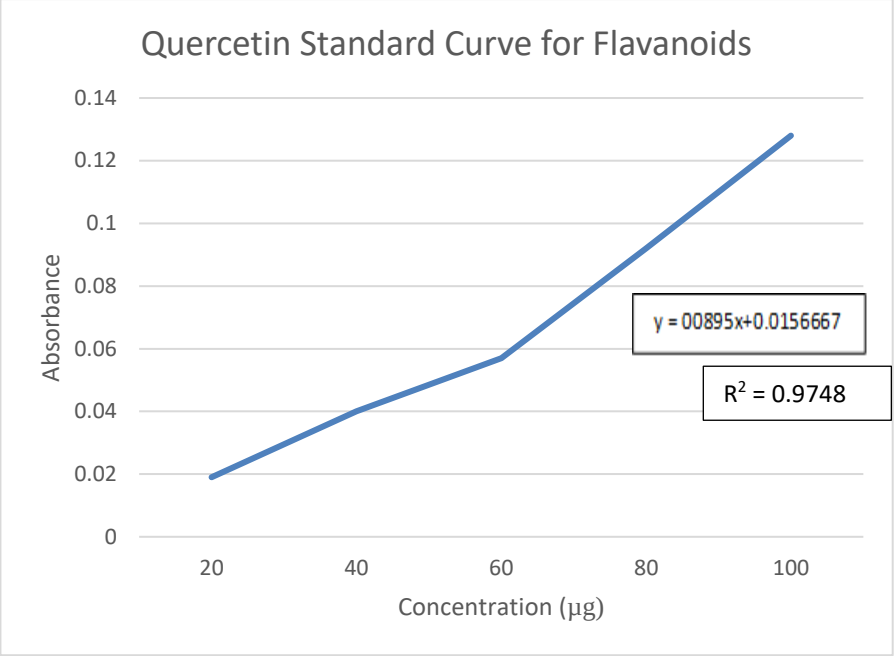

Fig.3 Quercetin standard curve for flavonoid content

Table 2: Antibacterial activity of Ethanolic extracts of *Carthamus tinctorius L.* by disc diffusion test

Test Microorganisms	Ampicilin	Amphotericin	Streptomycin	Plant Extract	Control
	Inhibition Zone in mm				
Klebsiella pneumoniae	21.1 ±1.386	9.2 ±1.686	R	9.4 ±1.818	_
Pseudomonas aeruginosa	17.2 ±0.62	R	R	29.6 ±1.818	_
Staphylococcus aureus	R	R	R	18.1 ±1.386	_
(R=Resistance, -Negative)					

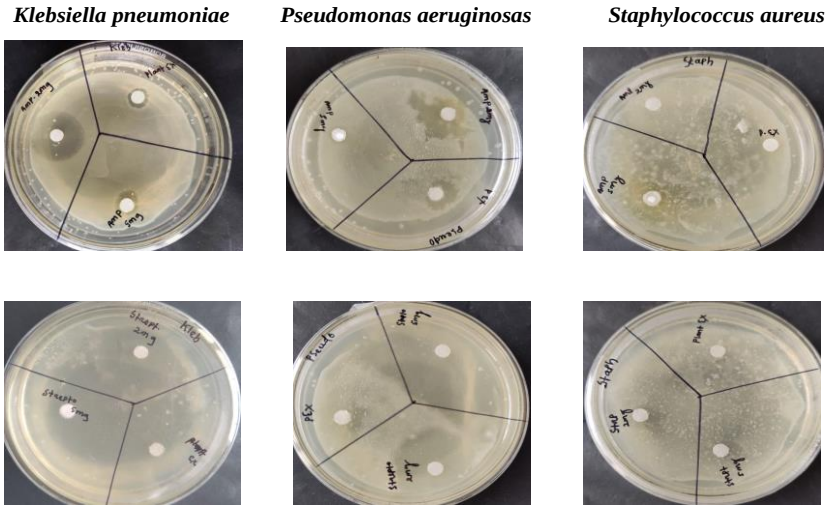

Fig.4 Plant extract showing inhibition zone against test organisms

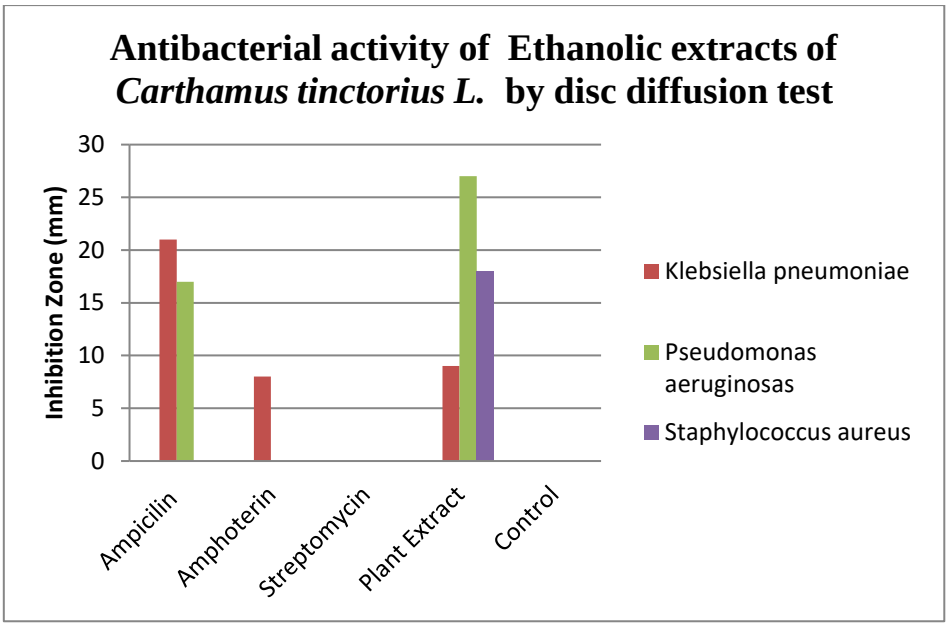

Figure 5: Comparative response of the bacterial strains to common antibiotics and ethanolic extract of *Carthamus tinctorius* L.

Antibacterial Activity of Extracts:

The *Carthamus tinctorius* L. plant extracts demonstrated antibacterial activity with inhibitory zone widths ranging from 9.4 ±1.818 mm to 29.6 ±1.818 mm. As positive controls, amphotericin, streptomycin, and ampicilin discs produced inhibitory zones. As negative controls, ethanol and distilled water were chosen since they did not induce zones of inhibition. Table 2 displays the antibacterial activity of *Carthamus tinctorius* L plant extracts. The ethanolic extract of *Carthamus tinctorius* L, had the most efficacy against *Pseudomonas aeruginosa* (29.6 ±1.818 mm) and *Staphylococcus aureus* (18.1 ±1.386 mm) and the efficacy against *Klebsiella pneumoniae* (9.4 ±1.818 mm) as inferred from the inhibition zones produced.

Thus, the study indicates that apart from having antibacterial properties, the leaves of *Carthamus tinctorius* L. are rich in flavonoids, phenolics, and tannins. The concentration of Phenolics was found to be highest (173.945 ± 2.214 µg GAE/ mg Plant Extract), followed by Tannins (49.486 ± 2.06) µgGAE/mg Plant Extract and Flavanoids 11.552 ± 0.335 µg Quercetin Equivalent /mg Plant Extract).

Conclusion

In conclusion, the findings of this study shed light on the rich phytochemical composition of *Carthamus tinctorius* L. leaves collected from Chhattisgarh, highlighting their potential as a valuable source of natural antioxidants. The presence of flavonoids, phenols, and tannins underscores their significance in the development of nutraceuticals and pharmaceuticals. These compounds exhibit promising therapeutic properties, including antibacterial activity and the potential for treating various diseases. However, further research is necessary to establish the quality, effectiveness, and safety of *Carthamus tinctorius* L. as a therapeutic agent.

To harness the full potential of *Carthamus tinctorius* L., future studies should focus on conducting extensive pharmacological and chemical experiments, as well as investigating human metabolism. Identifying and isolating the active constituents responsible for the observed pharmacological activities will contribute to a more comprehensive understanding of the plant's therapeutic properties. Additionally, it is crucial for clinicians to exercise caution until more definitive studies are conducted. This research serves as a foundation for further investigations into the application of traditional medicinal plants, emphasizing the need for continued exploration of *Carthamus tinctorius* L. as a potential source of new therapeutic drugs. By expanding our knowledge in this field, we can unlock the untapped potential of natural remedies and contribute to the development of innovative treatments for various ailments.

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