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Research Article

In Vitro Antibacterial Activity and Phytochemical Screening of Leaf Extracts of Erythrina Species

Feleke Worku [1*], Abrham Biresaw [2]

[1] Debre Markos University, College of Natural and Computational Sciences, Department of Chemistry, Ethiopia.

[2] Injibara University, College of Natural and Computational Sciences, Department of Chemistry, Ethiopia.

***Corresponding Author:** Feleke Worku, Debre Markos University, College of Natural and Computational Sciences, Department of Chemistry, Ethiopia.

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Abstract

The Present work was conducted to screen type of phytochemical constituents present in various extracts of Erythrina species leaves. Phytochemical of species were investigated for the presence of bioactive compounds. The phytochemical screening was conducted using water, chloroform, petroleum ether ethyl acetate and methanol extract of the plant. The study for antibacterial activity of leave extract of Erythrina species was conducted against Gram positive bacteria of Bacillus aereus and Gram Negative of Escherichia coli, Pseudomonas aueroginosa and Klebsiellapneumonia by using plate agar diffusion method that was employed to assess the antimicrobial activity of the prepared extract. The plant extracts were screened for the presence of alkaloids, glycosides, saponins, phytosterols, triterpens, phenols, tannins, flavonoids, diterpenes, anthraquinones,coumarin and Steriodal. This confirmed that Erythrina species leaves were the main sources of secondary metabolites. The extracts showed significant antibacterial activity in all gradient solvents.

Key words: erythrina species; phytochemical; antibacterial activity; bioactive compounds

1.Introduction

Since ancient times, people have been exploring large number of medicinal plants in the search of new drugs. [1-2]. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates bioactive principles and lead compounds in synthetic drugs [3-4]. One of the most important medicinal plants, which are widely used in the traditional system of medicine, is E.species [5]. This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable and safe [6]. This medicinal plantis useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [7]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are primary constituents and secondary compounds have terpenoid, alkaloid and phenolic compounds [8]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [9]. Erythrina species are trees, shrubs and a perennial herb with trifoliated leaves [10-11]. It is commonly called Leguminoseae which belongs to the genus Erythrina comprising of about 110 species of trees and shrubs. The name coral tree is used as a collective term for this plant. It is a tall evergreen tree native to savannah of tropical Africa [10]. The genus is widely distributed in tropical and subtropical areas of the world. It is typically found on sandy soil in littoral forest up to 250m in elevation. The Erythrina tree is cultivated especially as an ornamental tree and as a shade and soil improvement tree for other tree crops such as cotton, coffee and cacao. This plant produces many secondary metabolites, some of which have a function of defense systems against pathogenic bacteria [12-18]. Erythrina species are used as healing agents in traditional medicine in Africa. Of 31 African species, 35% have ethnomedical uses in sub-Saharan Africa. These are E. abyssinica, E. addisoniae Harms, E. excelsaBak, and E. fuscaLour, Other species may be used for health care [19-20].E. species grows in Ethiopia is recognized as subspecies where it is known locally as koreche (Amharic) and buri (Agewugna).In Ethiopian traditional medical practices, the leaves extracts of E. species are

administered orally for treating abdominal pain, intestinal worms water borne diseases, cancer, blood pressure and fungal diseases which are treated with these decoctions. Hence, the focus of this research is to undertake the phytochemical screening of the plant and evaluate the antimicrobial properties of the leaf extract of E. species using with suitable (different organic) solvents such as chloroform, petroleum ether, Ethyl acetate, Methanol and water.

Figure 1.1: Erythrina species plant [January, 2019

2. Materials and Methods

2.1Materials

2. 1. 1 Collection and identification of plants materials

The Fresh Leaves of Erythrina species were collected in October, 2019 from Amhara Region, Awi Zone, Injibara sub-city, Tekeluseta Local Kebele which is located in 527km from Addis Ababa, and capital of Ethiopia in the North-Eastern part of the country. The plant material was identified and authenticated by Mr. Mark MacLachlan botanist in SIM forestry project study, Awi Zone, Injibara, Ethiopia, where voucher number (ML/1992) was given and specimens were deposited.

2. 2. Methods

2.2.1. Preparation of plant leaf extracts

Plant material collected were carefully washed under running tap water followed by sterilized distilled water, and was air dried at room temperature in laboratory for 45 days. The dried plant material was then homogenized to a fine Coarse powder using an electric blender and then stored in air tight containers until further use.150gm of homogenized powder of plant material was soaked in different conical flasks containing 500 ml of water, ethyl acetate , petroleum ether, methanol, and chloroform were allowed to stand at least for 48 hours. Each organic solvent bath was occasionally, which was then kept on rotary shaker at 200rpm for 48h [8-9]. Finally samples of extracts (water, ethyl acetate, petroleum ether, methanol and chloroform) were prepared by using Soxhlet apparatus and was filtered through sterilized Whatman No 1 filter paper and concentrated to dryness under vacuum at 400C using rotary evaporator. Thus the obtained dried extracts were stored at 40C in labeled and stored in sterile bottles [10-11].

2. 2. 2. Phytochemical screening the extract for bioactive agents

Phytochemicals are the chemicals that present naturally in plants. Now-a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as manfriendly medicines. The qualitative phytochemical screening examinations were done to determine the class of compounds present in the crude leaves extract Erytrina species following the standard protocols [21-26].

1.Alkaloids: Extracts were dissolved in dilute Hydrochloric acid and filtered.

a) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids

b) Hager'stest: A small amount of Hager's reagent is added to the extract. The formation of yellow precipitate indicates the presence of alkaloids [14].

1. Glycosides (Modified Borntrager's Test): Extracts were hydrolysed with dilute HCl, and then subjected to test for glycosides. Extracts were treated with FeCl3 solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

2. Saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test:-0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

3. Phytosterols (LibermannBurchard's test): Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. On addition of Concentrated Sulphuric acid, formation of brown ring at the junction indicates the presence of phytosterols.

4. Triterpenes (Salkowski's Test): Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

5. Phenols (Ferric Chloride Test): Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Tannins (Gelatin Test): To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Diterpenes (Copper acetate Test): Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

9. Anthraquinones: About 0.5 g of the extract was boiled with 10% HCl for few minutes in water bath and filtered. The filtrate was allowed to cool and equal volume of CHCl3 was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. The formation of rose-pink color was taken as an indication for the presence of anthraquinones.

10. Coumarins: 3 ml of 10% NaOH was added to 2 mL of extract formation of yellow colour indicates the presence of coumarins.

11. Steroids (Salkowski test): When about 0.2 g of the extract was mixed with 2 mL of chloroform and 3 mL of concentrated sulphuric acid, appearance of red color at lower layer indicates presence of steroids and formation of yellow colored lower layer indicates presence of triterpenoids.

2. 3. Antimicrobial investigation

2.3. 1. Antimicrobial activity test

Microorganism Strain: The antibacterial activity of extracts of Erythrina species were evaluated by using four different bacterial strains: Bacillus aereus which is Gram-positive bacteria and, Escherichia coli, Klebsiella pneumoniae, P. aeruginosa and Escherichia coli which were Gram-negative to the microorganisms. These microorganisms were cultured in microbiology laboratory of biology department of Debre Markos University, Ethiopia.

Evaluation of Antibacterial Activity: Dried leave powders were extracted using different solvents (petroleum ether, ethyl acetate, chloroform, methanol and water). The antibacterial activity of leave extract of Erythrina species tests were carried out by Cup plate technique [27- 29]. It is performed in the accordance with the guidelines of National Committee for Clinical Laboratory Standards 9, with minor modification. The sterile nutrient agar plates were prepared. The bacterial test organisms like Escherichia coli, P. aeruginosa, Klebsiellapnemoniae, and Bacillus aereus were spread over the nutrient agar plates by using separate sterile cotton buds. After the microbial lawn preparation three different extracts of plant disc were placed on the organism inoculated plates with equal distance control discs were also prepared. All bacterial plates were incubated at 27°C for 24 hrs. A paper disc impregnated with Teracycle (50mg/mL) was used as positive control. The diameter of the minimum zone of inhibition was measured in mm as indicated (Table 3). For each test, three replicates were performed [31].

The Screening of extract for antibacterial activity byCup plate method:

Antimicrobial activity was expressed as the average diameter of the inhibition zones of three replicates.

The inhibition zone (IZ) in each case was recorded and the activity index (AI) was calculated as compared with those of their standard reference drugs using AI = X1 / Y1 where

X1= inhibition zone of test sample Y1= inhibition zone of standard

3. Results and Discussion

3.1. Extractive value and percentage yield

The yield of dried extracts was calculated according to the following algebraic equations:% Yield = the weight of extract ×100 / the weight of the powder The yield of sequential extracts (in gm.) is shown in Table 1 below. Percentage yields of 10.7 %, 9.3%, 12.7%, 12 %, and14% (w/w) of the E. species leaves were obtained by the maceration method of the plant extraction of 150 g of the plant materials. The extractive value of leave powder in various gradient solvent system showed that the methanol extraction has (14 %) which is the highest yield followed by ethyl acetate extraction (12.7%), water extraction (12%), chloroform extraction (10.7%)and petroleum ether extraction (9.3%)

Sr. No	Solvent	Color of extract	Mass of extract (g)	% yield (w/w)
1	Chloroform	Yellowish	18	10.7%
2	Petroleum ether	Brownish	14	9.3%
3	Ethyl acetate	Brownish	19	12.7%
4	Water	Dark brown	16	12%
5	Methanol	Brownish	21	14 %

Table 1: Extractive values of leave extracts of Erytrina species

3.2. Phytochemical screening results

The phytochemical analysis of plant extracts using petroleum ether, chloroform, ethylacetate, methanol and water are shown in Table 2. As indicated in the table, methanol extract shows all positive tests for the presence of different phytochemicals except diterpenes which was followed by chloroform and ethyl acetate extract where most of results were detected positive except phytosterols, diterpenes, anthraquinones and saponins, phenols and anthraquinones respectively while in petroleum ether and water extracts it is observed that there is 66.67% of positive results and 33.33% of negative result for testing of phytochemical compounds. From phytochemical analysis of experimental data, alkaloids, coumerins and steroids were present in all gradient solvent extracts except that of water. Alkaloids were present in all the solvents' extracts except water extract of E.species which it is evidently absent.

Sr.No	Phytochemical Constituents	Leaves Extract				
		Petroleum ether	**Chloroform**	**Ethyl acetate**	**Methanol**	**Water**
1	Alkaloids Wagners Test Hager's reagent	 + +	 + +	 + +	 + +	 - -
2	Glycosides	+	+	+	-	+
3	Saponins	+	+	-	+	+
4	Phyto sterols	-	-	+	+	-
5	Triterpenes	+	+	+	+	+
6	Phenols	+	+	-	+	+
7	Tannins	+	+	+	+	+
8	Flavonoids Alkaline Reagent Test Lead acetate Test	 - -	 + +	 + +	 + +	 + +
9	Diterpenes	-	-	-	-	+
10	Anthraquinones	-	-	-	+	+
11	Coumerins	+	+	+	+	-
12	Steroidal	+	+	+	+	-

Where; +indicates present, - indicates absent

Table 2: Qualitative phytochemical analysis of aqueous extract of leaf of Erytrina species

Glycosides were present in all the solvents' extracts except in methanol leave extracts of the plant which it is glaringly absent. The chloroform extract confirms the presence of alkaloids, Glycoside, saponins, triterpens, phenols, tannins, flavonoids, coumerins and steroidals. However, water extract showed the presence of Glycoside, saponins, triterpens, phenols, tannins, flavonoids, diterpenes and anthraquinones. Petroleum ether extract gave positive result for the presence of alkaloids, glycosides, Saponins, triterpens, phenols, tannins, coumerins and steroids. Diterepenes were observed only in water extract of the leaves of plant. Ethyl acetate and methanol extracts contained in common alkaloids, phytosterols, tannins, flavonoids, coumerins and steroids of secondary metaboliccompounds. Phytochemical analysis shows that alkaloids, glycosides, saponins, phytosterols, triterpens, phenols, tannins, flavonoids, diterpenes, anthraquinones, coumarin and steriodalare presented in leaves of E. species. In the present experiment it was observed that the amount of phytochemicals was higher in methanol extracts and less amount of biochemical was found in petroleum ether and water extracts. The result highlighted the significance of E. speices as a cheap source of bioactive traditional drugs for the rural and tribal people. There is a need to explore more E. species that will add new dimensions toward traditional management and conservation of plant wealth.

3.3 Antibacterial activity tests

The results of antimicrobial screening of leave extracts are shown in Table 3 below. The results revealed that activities of the sequential leave extracts against selected gram positive and gram negative test microorganisms. The antibacterial activity in terms of zone of inhibition (in mm diameter) of petroleum ether, chloroform, ethyl acetate, methanol and water extracts of E. species leaves at the different concentrations of against four pathogenic organisms Bacillus subtilis, Escherichiacoli, Pseudomonasaeruginosa and Klebsiellapneumonae. The activity of extracts has also been compared with the broad spectrum commercially available antibiotic (tetracycline). The results also showed that water, ethyl acetate and methanol extracts were the best solvents for extracting antimicrobial substances from this plant compared to petroleum ether and chloroform extracts. The Pseudomonas aeruginosa was resistant to water and petroleum ether extract while Bacillus subtilis was resistant to only petroleum ether extract. Pseudomonas aeruginos is highly sensitive to methanol. Klebsiella pneumonia was resistant to water and ethyl acetate extract. Klebsiella pneumonia was highly sensitive to chloroform extract.

solvent		Antibacterial Activity of Erytrina species leave extracts (MIC in mm)					
	Bacillus subtilis		**E. coli**		**Pseudomonas aeruginosa**		**Klebsiella pneumoniae**	
	*ZI (mm)**	*AI***	*ZI (mm)*	*AI*	*ZI*	*AI*	*ZI (mm)*	*AI*
Water	30	0.9	14	0.5	16	0.5	-	-
Chloroform	15	0.5	17	0.6	13	0.4	27	0.9
Petroleum Ether	-	-	19	0.6	-	-	12	0.4
Methanol	20	0.6	24	0.8	33	0.9	22	0.7
Ethyl acetate	24	0.8	15	0.5	14	0.4	-	-
Tetracycline 50mg /ml	32	-	30	-	35	-	30	-

*Zone of mean inhibition of bacteria, **Activity index

Table 3: Antibacterial Activity of leaf extract of Erytrina species in different solvents

The maximum activity of methanol extract was seen against Pseudomonas aeruginosa at concentration of 33mg/ml.

The minimum activity of petroleum ether extract was seen in Klebsiella pneumonia at concentration of 30mg/mL. The maximum activity of water and ethyl acetate extract was seen against Bacillus subtilis at concentration of 32mg/ml. The maximum activity of chloroform extract was seen against Klebsiella pneumonae at concentration of 30mg/mL as shown in Table 3. The plant extracts compared favorably with the standard antibiotic Tetracycline (Table 3). The chloroform leaves extract of E. species inhibited the growth of Escherichia coli with value of 24mm at the 30mg/ml.Water extract ofE. species leavesinhibited the growth of with value of 30mm at the concentration of 32mg/ml. Methanol extract of E. species leaves inhibited the growth of with value of 33mm at the concentration of 35mg/ml.

The methanol and water extracts exhibited much higher antibacterial activity against the tested pathogenic bacteria and had much higher concentration to the rest of bacterial strains. The chloroform and methanol fractions exhibited moderate antimicrobial activity in both bacterial species. Conversely, the petroleum ether and water extracts exhibited less activity against bacterial species. Drug resistance among bacterial species is a serious problem in public health thus the discovery and development of new antimicrobial drugs from plants are among the most exciting areas of pharmacological research [10]. The present study extends the efforts of discovering drug templates from Ethiopia medicinal plants. Extracts revealed that E. species contains secondary metabolites with antibacterial activity against Gram positive bacteria and Gram negative bacteria. Methanol and water extract E. speciesexhibited high antibacterial against all tested bacteria which implies that polar secondary metabolites are responsible for the activity. By the above results, it can be concluded that E. species can be a good source for antibacterial drug against various bacterial pathogens.

2. Conclusion

The present study indicated that E.species is a farm plant having various medicinal and pharmacological properties. The secondary metabolites obtained from extracted plant part are alkaloids, glycosides,saponin, flavonoids, tannin, coumerins, anthraquinones, steroids, triterpens, phytosterols,diterpenesand phenol.Because of the presence of these secondary metabolites the selected medicinal plants have high healing potential. These phytochemicals render the medicinal values of the studied plants. It is evident that the extract of E.species contains secondary metabolites which possess remarkable antibacterial activities. Thus, bactericides of great value could be developed from antimicrobial secondary metabolites from E.speciesas alternative medicines to manage pathogenic bacteria.

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Declaration

Ethical Approval

The fresh leaves of Erythrina species were collected from its natural habitat in October ,2019 Amhara State, Agewawi Adminstrative zone ,Injibara sub-city, Tekeluseta local area of Injibara (23O- 37'11 “N, 30O -26'4” E) Ethiopia. The plant material was identified and authenticated by Mr. Mark MacLachlan Botanist in SIM forestry project study, Injibara, Ethiopia, where voucher number (ML/1992) was given and specimens of Erythrina species were deposited at national Herbarium protocol.

Licenses And Permission

Much of Awi Administrative Zone natural habitat plants including Erythrina species are protected by legislation to ensure its survival and to protected biodiversity. All plants that are indigenous to Awi Administrative Zone, Amhara region, Ethiopia are protected by guidelines and legislation. The permission and licenses was given by Amhara National regional state forestry enterprise establishment council of regional regulation No. 70/2009. So it is applicable

Consent

It is not applicable.

Availability of data and materials:

The authors confirm that the data supporting the findings of this study is available with in the article or its supplementary materials. The data used

in this research is available in a public repository that issues datasets with DOIS when necessary.

Competing Interests

Authors declare that we have no competing interests.

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Authors' Contributions:

Feleke Worku developed the concept of the research protocol, study design, literature review, data analysis and interpretation, whereas the corresponding Author Abrahm Biresaw developed data collection, data extraction, and drafted the manuscript. The authors read and approved the finalized manuscript writing.

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