Research Article

Flavonoids, alkaloids, and saponins as antimicrobial agents from *Fragaria vesca* L.

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Abstract

Strawberry (*Fragaria vesca*) being a rich source of bioactive health-promoting compounds, has been given attention worldwide in the identification of functional foods. The high levels of phenols along with its resilient smell and sublime white pink color have compelled the scientists to termed it as a preferred berry. Future researches having the focus on a strawberry is proving the fact that it is the main product utilized in the preparation of standard food, jellies, jams, and beverages comprising alcohol content. The said fruits have been used traditionally in different drug systems for curing a wide range of diseases mainly as purgative, anti-diuretic, and anti-septic. The leaves of the same plant can be very helpful in urinary problems, diarrhea while the leaves have been used as anti-diarrheal, urinary, and antiseptic. The current research is focused to provide and discuss the available literature to first check the quality and quantity of flavonoids, saponin, and alkaloids contents in the fruits of strawberry. In this focused attempt, selected metabolites other than primary i.e. flavonoid alkaloid and saponin were quantified from the methanolic dry filtrate of strawberry which was lied open to anti-microbial, anti-oxidant, and other allopathic actions and were found active against these biological estimations. The overall results reveal that the test species can be a good source of natural medicinal and pharmaceutical drugs for many sorts of diseases and imbalances.

Keywords: Allelopathic; Antifungal; Antibacterial; Crude; Ethanolic; Extract; Effects; Strawberry (*Fragaria vesca*)

Introduction

Plants according to modern researchers are known as universal converters of energy deployed by the sun on the planet earth. The life of all organisms on planet earth depends upon Plants due to their vast range of applications like food, therapeutic purposes, clothing, flavoring agents, and mainly for ornamental purposes. Herbaceous plants renowned for medicines contain

phytochemicals of immense therapeutic outcomes and are very significant in curing different microbial illnesses [1, 2]. The knowledge and utilization of these herbs were present in different indigenous and outdated systems like Ayurveda, Unani, and some local systems of India which were mostly used for defensive and medicinal ailments. A similar system of folk medicine was present in China, it is the main

knowledge through which people get income throughout the world [3, 4].

The major proportion of the total population of the world (60-80%) is completely dependent upon medicinal herbs and utilize them as food, medicine, shelter, and defense purposes due to their cheapness, effectiveness, and easy availability [5, 6]. According to different surveys and calculations, the range beyond 35k plants is used for therapeutic purposes. Such a large amount of these plants reflect the fact that it comprises a vast variety of secondary chemical substances which mainly include alkaloid, saponin, flavonoid, tannin, and resins, etc. Almost 7,000 herbs of medicinal importance normally flourish in the Asian subcontinent [7, 8]. In Pakistan, almost 5520 plant species flourish in northern and southern mountainous ranges which contain almost 600 plant species recognized and utilizing as therapeutic drugs. Such a large extent of medicinal species can be termed as a gift from the Creator of the universe [9].

The genus *Fragaria* (strawberry genus), comes under the umbrella of the family Rosaceae commonly known as the rose family. The members of the family Rosaceae are semi-cosmopolitan in distribution and comprises more than 3000 species [10]. The sub-family *Rosadeae* contain the super-tribe Rosedale which further contains *Potentilleae* as a tribe and which further divides into sub-tribes, of which *Fragariinae* is a single sub-tribe [10, 11].

Rosacea family contains many plant species that are included in the list of either fruits or vegetables and are the main source of income and have economic benefits. They mainly include fruits like *Pyrus* spp commonly known as pears, *Fragaria* × *ananassa* known as strawberries, *Malus Domestica* for apples, *Prunus persica* for peach, and many other fruits and food giving plants. This number of plants having ornamental features make this family an important one [12, 13].

Considering the importance and economic benefits, *Solanaceae* acquires the first position followed by *Poaceae*, and the Rosacea family comes in third place [14, 15]. The genus *Fragaria* present in the family Rosacea comprises twenty-four (24) species [14]. The species count is relatively very low in comparison to the *rubus* genus which is comprised of about 745 species, its type specimen mainly includes black raspberry (*R. occidentalis*) and red raspberry (*R. idaeus*). The genus *Fragaria* ranges in their ploidy level from 2n to 10n. Considering all species, ½ of the total species are diploid, the prominent example includes *F. vesca*, which have seven pairs of chromosomes ($2n = 2 \times 7 = 14$), four species in the genus are octoploid ($2n = 8 \times 7 = 56$) the prominent species is *F. ananassa*. Tetra and octaploid have equal proportion of four ($2n = 4 \times 7 = 28$), one in total 24 species is hexaploid ($2n = 6 \times 7 = 42$) and *F. bringhurstii* exhibit different ranges comprising of $5n = 5 \times 7 = 35$ (Penta), 6n (Hexa) and 9n (nona) ploidy ($2n = 9 \times 7 = 63$) [16].

Materials and Methods

Antibacterial Assay

The evaluation of antibacterial activity was done by utilizing Agar Well Method. The efficacy of each selected bacterium was measured in ZOI (Zone of Inhibition) in mm following the Asghar *et al.* method [17] the growth medium comprising of nutrient agar (medium for bacteria growth) was prepared by a stranded procedure in which all the equipment utilized must be sterilized to prevent any other microbial growth. Media was prepared by taking 27g of nutrient agar and dissolved in 1L distilled water in a flask. The media was incubated at 121°C and 20 lb. pressure for 20 minutes in an autoclave to kill any kind of microbes present inside the media and to facilitate only the desired bacterium for growth. The media was then put out from the autoclave and placed at room temperature, when the temperature falls below 50 °C, 20 mL media was poured into

each plate (Petri dish) and for solidification of the medium, the plates were kept at room temperature and after which, the plates were stored at 0 °C in the refrigerator. Upon complete solidification, the bacterium was inoculated on the medium with the help of a spreader and kept at 37 °C for 24 hours inside an incubator which will facilitate bacteria to grow into colonies. The fresh bacterial growth was checked through a spectrophotometer. The reserved plates containing media were put out from the fridge and inoculum was spread throughout the media with the help of a spreader. Holes were made with the help of a borer with a specific distance in between holes. Different focused fractions and crude extracts were installed in the holes and plates were sealed and kept in an incubator for 24 hours at 37 °C. After 24 hours, inhibition zones were measured for each bacterium and their effect is observed and noted.

Antifungal assay

The assessment of different extracts against the fungus was determined by utilizing the method of Atta-ur-Rahman *et al* with some modification [18]. Potato dextrose broth (PDB) culture was prepared by taking 14g of PDB and dissolving it in 1 liter distilled water. After shaking, the media was autoclaved to remove any kind of germs and microbes present in it by heating it to 121°C and 20 lb. pressure for 20 minutes. The media was then placed at room temperature for gradual cooling, after which it was poured into four flasks of 250 ml capacity for separate inoculation of fungal strains and was placed inside a shaking incubator at 27°C and 190 rpm for 6-7days for maximum growth. While the inoculum was inside the incubator, a fresh media was prepared by mixing 27 g nutrient agar in 1 liter of distilled water and the same autoclaving process was repeated for this media as well for the same cause. After sterilization, 10 ml media were poured into each test tube and the inoculum in an

amount of 1 ml was added to each tube and was closed with the help of cotton slugs to prevent any dust or impurity. All the tubes were positioned in an inclined manner and fungus was applied in the inclined area of the media and the tubes were placed inside the incubator for 7 days and activity was checked. The zone of inhibition was measured in millimeters.

Activity for allopathic diagnosis

The fractions selected for diagnosing the allopathic activity were first prepared in-stock solutions, for which 1 gram of each fraction (i.e. crude methanolic extract, chloroform, n-hexane, and ethyl acetate) is dissolved in 6ml of DMSO in separate falcon tubes, which makes it 1% w/v solution. Five different concentrations were selected to check the activity at a different dosage. These concentrations were 3, 10, 30, 100, and 300 ppm for each stock solution.

Experimental method

For every experiment, a standard is compulsory which is utilized for comparing the activity of focused fractions. For this purpose, grains of *Oryza sativa* (rice) were utilized. Surface sterilization of the grains was done with ethanol and washed with water thrice to eradicate any possible germs. Sterile Petri dishes were taken in which (Whatman # 42) filter paper of equal size was placed and rice grains were placed on it for cultivation purposes. After germination, the seeds were distributed in each petri dish with an equal count of 10 per plate. Inoculum in an amount of 5 ml from each fraction was poured into each petri dish and was labeled for the specific fraction. After which the plates were kept at 20°C in an incubator having complete darkness for 7 days. The estimation of inhibition reflects the growth of root and shoot, the percentage of germination, and fresh dry weight. For each fraction, the following parameters were determined and compared to the 1% DMSO labeled as

control. The procedure was followed three times to get accurate results [19, 20].

Results and Discussion

For analyzing secondary metabolites, the fruits of strawberry were dried in shade and then ground to obtain a powdered crude extract, which was then placed in methanol and were kept for shaking at room temperature for 10 days, which were shaken regularly [21, 22]. The process was performed twice and the filtrate got condensed to $\frac{1}{4}$ of its original volume with the help of a rotary evaporator. The filtrate was almost 110g, which was then dried and both quantitative and qualitative analysis was done for flavonoids, saponins, and alkaloids. The analysis yield that flavonoids (2.3) were comparatively greater than the other two. The high amount of flavonoids reflects the better anti-microbial and anti-oxidant actions of strawberry fruits.

Anti-bacterial activity

The extracts obtained in crude form from strawberry fruits were used against bacterium permissible to check its action, the focused bacterial strains comprise of *S.aureus*, *E.coli*, *Bacillus subtilis*, *Xanthomonas Campestris*, and *Clavibacter Michiganensis* [23]. The extracts prevented the growth of all strains and the results were found to be significant.

Flavonoid

After isolating flavonoids from crude extract, it was employed against all strains to check its activity, which ultimately gives significant results against all strains. The highest inhibitory action was shown against *Xanthomonas campestris* with a 16 mm inhibition area followed by *Bacillus subtilis* and *S. Aureus* with 14 mm zones for each and was least active against *Clavibacter Michiganensis* and *E. Coli* with 12 mm zones respectively (Table. 1 & 2; Fig. 1 & 3).

Alkaloid

Alkaloids also after isolation were checked against the same five bacterial lines in which the results column was significant for a few while some of them remain unaffected by the application of the said metabolite. *Xanthomonas campestris* and *S. aureus* were prevented by the alkaloids up to 12 mm of inhibitory zones while *E. coli* was repressed up to 10 mm respectively (Table. 1 & 2; Fig. 1 & 3). But *Clavibacter michiganensis* and *Bacillus subtilis* flourished completely in the presence of the said alkaloid.

Saponin

Saponin on segregation from dried strawberry fruit was assessed against the five different species of bacteria utilized in this experiment. Similarly, as the results of alkaloids, Saponin also gave beneficial results against some bacterium and was unable to stop the growth of others. The highest activity of Saponin was shown against *S. Aureus* with a 16 mm repression zone followed by *Bacillus subtilis* with a 14 mm repression zone (Table. 1 & 2; Fig. 1 & 3). The remaining three bacterial species dwell normally although by the application of the mentioned metabolite. They comprise *Clavibacter Michiganensis*, *Xanthomonas campestris*, and *E. coli*. The above-mentioned results for all three secondary metabolites suggest that flavonoids are the most active amongst all of them against the same bacterial species [24].

Anti-fungal activity

Three fungus species were selected due to their abundance and their pathogenic nature and their growth was tested against Alkaloid, flavonoid and Saponin of strawberry fruit. All the mentioned metabolites showed activity against the fungus species. All the secondary metabolites were employed in 22 mg/ml while standard (Mancozeb) was taken in 2 mg/ml.

Table 1. Antibacterial Profile of *Fragaria vesca* L.

Name of the Samples	<i>Clavibacter Michiganensis</i> (mm)	<i>Xanthomonas Campestris</i> (mm)	<i>S. Aureus</i> (mm)	<i>E. coli</i> (mm)	<i>Bacillus subtilis</i> (mm)
Alkaloid 22mg/mL	0 ± 0.00	12 ± 0.42	12 ± 0.53	10 ± 0.53	0 ± 0.46
Saponin 22mg/mL	0 ± 0.31	0 ± 0.31	16 ± 0.42	0 ± 0.37	14 ± 0.27
Flavonoid 22 mg/mL	12 ± 0.38	16 ± 0.37	14 ± 0.63	12 ± 0.48	14 ± 0.48
Antibiotic 2mg/mL	24 ± 0.42	28 ± 0.67	30 ± 0.36	26 ± 0.49	22 ± 0.33
DMSO	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00

Table 2. ANOVA: Two-Factor Antibacterial Potential *Fragaria vesca* L.

Anova: Two-Factor Antibacterial						
Summary	Count	Sum	Average	Variance		
Alkaloid 22mg/mL	3	24	8	48		
Saponin 22mg/mL	3	16	5.333333	85.33333		
Flavonoid 22 mg/mL	3	42	14	4		
Antibiotic 2mg/mL	3	82	27.33333	9.333333		
DMSO	3	0	0	0		
<i>Clavibacter Michiganensis</i> (mm)	5	36	7.2	115.2		
<i>Xanthomonas Campestris</i> (mm)	5	56	11.2	139.2		
<i>S. Aureus</i> (mm)	5	72	14.4	114.8		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1313.6	4	328.4	16.09804	0.00068	3.837853
Columns	130.1333333	2	65.06667	3.189542	0.095815	4.45897
Error	163.2	8	20.4			
Total	1606.933333	14

Figure 1. Dynamics of the Antibacterial Profile of Alkaloid, saponin and Flavonoids

Figure 2. Dynamics of the Antifungal Profile of Alkaloid, Saponin, and Flavonoids

Figure 3. Antibacterial activity of Flavonoids, alkaloids, and saponins of *Fragaria vesca*.

Flavonoid

The most abundant secondary metabolite found in fruits of strawberry can be termed as flavonoids, which are act as powerful chemical agents are employed against the growth of the selected fungus species. The results were found to be good as it prevented the growth of all fungus up to certain limits [25]. The highest prevention was recorded for *Fusarium oxysporum* with 12 mm zone followed by *Aspergillus Niger* and *Rhizopus* with 10 mm zones (Table. 3 & 4; fig. 2).

Fungal growth against alkaloid, flavonoid, and Saponin obtained from strawberry fruits (measured in mm).

Alkaloid

The said metabolite although occurs to be less abundant in fruits of strawberry, but was still isolated and analyzed to check its action against focused species of fungi. The highest strength of repression was recorded against two different fungi with 12mm repression zones. It includes *Rhizopus* and *Aspergillus Niger* but less efficacy was shown against *Fusarium Oxysporum* with 10 mm

prevention zone (Table 3 & 4; Fig. 2). But the aggregate observation can be considered significant.

Saponin

The growth of selected fungus against Saponin was checked to obtain results that can be helpful in the formulation of cheap and efficient drugs in the future. Saponin was

found to be active against all fungal species in which the highest action was exhibited against *Fusarium oxysporum* with a 14 mm inhibition zone followed by *Aspergillus Niger* with a 12 mm zone and least activity was shown against *Rhizopus* with 10 mm zone respectively (Table 3 & 4; Fig. 2).

Table 3. Antifungal Profile of *Fragaria vesca* L.

Sample Name	<i>A. Niger</i> (mm)	<i>F.oxysporum</i> (mm)	<i>Rhizopus</i>
Alkaloid	12 ± 0.32	10 ± 0.28	12 ± 0.51
Saponin	12 ± 0.45	14 ± 0.20	10 ± 0.41
Flavonoid	10 ± 0.55	12 ± 0.43	10 ± 0.62
Mancozeb (standard)	24 ± 0.27	28 ± 0.36	30 ± 0.32
DMSO	0 ± 0.00	0 ± 0.00	0 ± 0.00

Table 4. ANOVA: Two-Factor Antifungal Profile of *Fragaria vesca* L.

Anova: Two-Factor Antifungal						
Summary	Count	Sum	Average	Variance		
Alkaloid	3	34	11.33333	1.333333		
Saponin	3	36	12	4		
Flavonoid	3	32	10.66667	1.333333		
Mancozeb (standard)	3	82	27.33333	9.333333		
DMSO	3	0	0	0		
<i>A. Niger</i> (mm)	5	58	11.6	72.8		
<i>F.oxysporum</i> (mm)	5	64	12.8	101.2		
<i>Rhizopus</i>	5	62	12.4	118.8		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	1142.933	4	285.7333	80.86792	1.66E-06	3.837853
Columns	3.733333	2	1.866667	0.528302	0.608833	4.45897
Error	28.26667	8	3.533333			
Total	1174.933	14				

Conclusion

in conclusion from the above results, discussions, and statistical analysis, it is very clear that the Flavonoids, saponin, and Alkaloids have significant activities against some bacterial and fungal strains but not all, and Flavonoids were the most effective antimicrobial biochemical in *Fragaria vesca* L.

Authors' contributions

Conceived and designed the experiments: SA Hussain & S Ahmad, Performed the

experiments: SA Hussain & KU Rehman, Analyzed the data: ZA Butt, Contributed materials/ analysis/ tools: SS Khan & S Ullah, Wrote the paper: KU Rehman, S Ullah & SS Khan.

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