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Research Article

ANTIMICROBIAL EFFICACY OF SOLVENT EXTRACTS OF SEEDS, FRUIT PULP AND LEAVES OF GRAVIOLA (GISHTA)

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ABSTRACT

The knowledge of plants as therapeutic agents is as old as disease, which in turn is as ancient as man himself. Since times immemorial plants have been in use to cure the human ailments. In traditional medicinal system, several plants have provided important cues for being used as potent bioactive compounds against various disorders and diseases causing organisms. al and Still number of plants has yet to be screened for their potential as medicinal plants. In accordance with this information, antimicrobial activities of plant were tested, which is commonly considered as traditional medicinal plant. Solvent extracts of leaves, fruit pulp and seeds of Graviola (Gishta) cultivar of Ethiopia made in petroleum ether, chloroform, ethanol, methanol were tested for antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Corynebacterium diphtheria, Xanthomonas citrovorum, Proteus vulgaris*, *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus fumigatus* and *Candida albicans*. The petroleum ether and ethanol extracts of seeds of Graviola or Gishta (Annona spp. of Ethiopia) shown increased zones of inhibition for the test bacteria *Corynebacterium diphtheria,* the fungal organism highly sensitive to methanol and chloroform extract was *Asperigillus niger.* The petroleum ether, chloroform and methanol extract of fruit pulp and leaves of Graviola or Gishta (Annona spp. of Ethiopia) shown increased zones of inhibition against fungal spp. The ethanol extract exhibited high activity against the growth of *Corynebacterium diphtheria*.The obtained results data statistacally analyzed and tested using a full factorial Analysis of Variance (ANOVA) model.

Keywords: Graviola (Gishta), Antimicrobial, Antibacterial, Antifungal and full factorial.

I. INTRODUCTION

The use of plants as herbal drugs is as old as human civilization itself. Many of the existing medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and other alternative medicinal systems have been utilizing plants as effective medicines to cure many harmful diseases. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world[1]. Graviola is a small, upright evergreen tree, 5–6 m high, with large, glossy, dark green leaves. It produces a large, heart-shaped, edible fruit that is 15–20 cm in diameter and green in color, with white flesh inside. The leaves, fruit, seeds, bark, root and stem are used to make medicine[2]. Synonyms: *Annona bonplandiana, A. senegalensis, A. cearensis, A. cherimola, A, macrocarpa, A. muricata, Brazilian cherimoya* and *Guanabanus muricatus*[2]. Graviola is a fruit that generally grows in the rain forests of Africa, South America, and Southeast Asia. It has other names like thorny custard apple, cherimoya and brazilian pawpaw. In various languages, this fruit is referred as: Gishta (Ethiopia), guanabana (Spanish), corossol (French), aluguntugui (Ghana), sorsaka (Papiamento), adunu (Acholi), guyabano, guanavana, durian benggala, nangka blanda, sirsak, toge-banreisi, nangka londa, zuurzak, Brazilian Paw Paw, Corossol, Corossol

Épineux, Corossolier, Durian Benggala, Guanabana, Guanavana, Nangka Blanda, Nangka Londa, Soursop, Sour Sop, Toge-Banreisi. guanaba, épineux, huanaba, toge-banreisi, durian benggala and cachiman épineux. In India, it is less known as shul-ram-fal and hanuman fal, and as mullaatha in Malayalam, whereas in Harar (Ethiopia) in Harari language known for centuries as *Amba Shoukh* (Thorny Mango or Thorny Fruit). "yebere lib" meaning heart of cow in Jimma, Ethiopia[3]. All parts of the Graviola (Annona spp.) tree have been used in traditional folkloric medicine in various cultures for various ailments and complaints. The leaves and seeds of the tree have long been used by native people of various cultures for an astounding variety of ailments, ranging from parasitic infections (the seeds) to high blood pressure and cancer. Seeds are emetic, Pulverized seeds and seed oil are effective against head lice. Flowers are regarded to be antispasmodic and pectoral and used to alleviate catarrh. Leaf Infusions have been used as emetic, sudorific, antispasmodic, antipyretic, insecticidal, eczema, skin eruptions, swollen feet and compresses for inflammation. Ripened fruit is reported as antiscorbutic and is also used as an anthelmintic. Pulp of soursop has been used as diuretic, hematuria and urethritis. The unripe and dried fruit and seeds used as astringent, used in diarrhea and dysentery. The fruit juice is used medicinally to treat illness ranging from stomach ailments to worms. The bark is used in powdered form for diarrhoea and dysentery. The fruit is also used as bait in fish traps. The bark has been used in tanning and the wood is a potential source of paper pulp[4]. To our knowledge as per the prvious literature, the Gishta or graviola (Annona spp.) extracts of Ethiopia has not been investigated for its biological activities. The principle aim of the work was to study the antimicrobial activity of graviola (Annona spp.) extracts in different solvents such as ethanol, chloroform, methanol and petroleum ether against seven species of bacteria and three species of fungi. In the experimental study different fractions of solvent extracts of leaves, fruit pulp and seeds of the plant have been investigated.

II. MATERIALS AND METHODS

Plant collection, identification and authentication

Graviola (Annona spp.) leaves and fruit(s) of Ethiopia were collected from Oromeo region, Ethiopia, in January 2014. The plant was identified, authenticated and supplied by Prof Behailu Etana Disasa of Natural Resource Management, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia.

Extract Preparation

Fresh fruit material was dried at room temperature (28 ±3^{0}C) by exposing to atmosphere under aseptic conditions. The dried materials were pulverized into a very fine powder. Ten grams of each sample was Soxhlet extracted sequentially using petroleum ether, chloroform, methanol and ethanol at 60^{0}C temperature[5]. The extract was dried in a flash evaporator for 30min and the left over powder was considered 100%. Different concentrations such as 250, 500, 750 and 1000 µg/ml were prepared by redissolving the extract powder in the same solvent which was used in the extraction.

CHEMICALS USED

Ethanol, chloroform, methanol and petroleum ether (SD Fine Chemicals, Mumbai) were used during experimental protocol.

Test organisms

Escherichia coli (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Corynebacterium diphtheria* (ATCC 75415)., *Xanthomonas citrovorum* (ATCC 8082), *Proteus vulgaris* (ATCC 638), *Staphylococcus aureus* (ATCC 25923), *Aspergillus niger* (NCIM 596), *Aspergillus fumigatus* (NCIM 291) and *Candida albicans* (NCIM 670) obtained from the department of pharmaceutical microbiology and biotechnology, College of Health Sciences, Aksum university, Axum, Ethiopia, NE Africa (**Characteristics are listed in Table No. 1**). All the above test bacterial species were maintained on nutrient agar medium. 36h old bacterial culture was inoculated into nutrient broth and incubated on a rotary shaker at 35 ± 2°C at 100 rpm. After 36h of incubation, the bacterial suspension was centrifuged at 10000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1×10^8 cfu/ml using UV Visible Spectrophotometer. By reading the OD of the solution to 0.45Å (610nm) it was used for further studies. Fungal colonies were harvested from 9 -10days old cultures, which were maintained on potato dextrose agar. The spores were suspended in sterile distilled water and the spore suspensions were adjusted to 1×10^8 spores/ml[6].

Antimicrobial assay

Different concentrations of solvent extracts of the seeds, fruitpulp and leaves were tested for antimicrobial activity by using antibiotic

sensitivity test[7&8]. Microbial suspension was evenly mixed with sterile agar medium and poured into the sterile Petri plates. After allowing the media to solidify at room temperature, wells of 6mm diameter were bored in the agar with sterile cork borer. Each concentration was checked for antimicrobial activity by introducing equal amounts of the sample (40ul) into wells. The method was repeated in five plates. Plates were allowed to stand at room temperature for 1 hour, for extract to diffuse into agar media and then incubated at 37°C for 24 to 48 hours. The zone of growth inhibition around the wells was measured and diameter of inhibition zone was calculated. Simultaneously the activity of standard antibiotics such as Streptomycin (10µg/ml) for bacteria, Nystatin (10µg/ml) for fungi under studying in similar conditions so as to compare the degree of inhibition by the solvent extracts. Agar wells fed with corresponding solvents served as control minimum inhibitory concentration which was determined as the lowest concentration on solvent extracts inhibiting the growth of organisms and was determined based on the readings. **(Table 1: List of the selected test organisms (Bacteria and Fungi))**

III. RESULTS AND DISCUSSION

RESULTS

Table No: 2 indicates the Ether extracts of seeds of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms, the minimum inhibitory concentration was found to be 250 µg for all tested bacteria such as *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Corynebacterium diphtheria, Xanthomonas citrovorum*, *Proteus vulgaris* and *Staphylococcus aureus*, while it was 500µg against *Candida albicans*. The remaining two fungi were controlled at 750µg. Among the microbial species tested *Corynebacterium diphtheria* was found to be highly sensitive to the ether extracts of seeds of Graviola or Gishta. The Chloroform extracts of the seeds of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be *500* µg for *Corynebacterium diphtheria* and *Candida albicans,* while it was 750 µg for *Escherichia coli*, *Bacillus subtilis, Pseudomonas aeruginosa, Xanthomonas citrovorum, Proteus vulgaris, Staphylococcus aureus, Asperigillus niger* and *Asperigillus fumigatus.* Among the microbial species tested, *Asperigillus niger* was found to be highly sensitive to the chloroform extracts of seeds of Graviola or Gishta. The Ethanol extracts of the seeds of Graviola or Gishta are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 500 µg for *Escherichia coli, Bacillus subtilis, Corynebacterium diphtheria, Proteus vulgaris, Staphylococcus aureus* and *Candida albicans,* while it was 750 µg for *Pseudomonas aeruginosa, Xanthomonas citrovorum, Asperigillus niger* and *Asperigillus fumigatus.* Among the microbial species tested *Corynebacterium diphtheria* was found to be highly sensitive to the ethanol extracts of seeds of Graviola. The Methanol extracts of the seeds of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 250 µg for *Asperigillus niger*, *Asperigillus fumigatus* and *Candida albicans,* while it was 500µg for *Escherichia coli, Bacillus subtilis, Corynebacterium diphtheria* and *Staphylococcus aureus.* The remaining three bacteria exhibited zones of growth inhibition at 750 µg. Among the microbial species tested *Asperigillus niger* was found to be highly sensitive to the methanol extracts of seeds of Graviola. **(Table 2: Inhibitory activity of solvent extracts of seeds of Graviola or Gishta).**

Table No: 3 shows the Ether extracts of fruit pulp of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms, the minimum inhibitory concentration was found to be 250 µg for all tested bacteria such as *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Corynebacterium diphtheria, Xanthomonas citrovorum*, *Proteus vulgaris* and *Staphylococcus aureus*, while it was 750µg against all the fungi. Among the microbial species tested *Corynebacterium diphtheria* was found to be highly sensitive to the ether extracts of fruit pulp of Graviola or Gishta. The Chloroform extracts of the fruit pulp of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be *500* µg for *Candida albicans,* while it was 750 µg for all the test organisms. Among the microbial species tested, *Asperigillus niger* was found to be highly sensitive to the chloroform extracts of fruit pulp of Graviola or Gishta. The Ethanol extracts of the fruit pulp of Graviola or Gishta are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 500µg for *Escherichia coli, Bacillus subtilis, Corynebacterium diphtheria, Proteus vulgaris, Staphylococcus aureus* and *Candida albicans,* while it was 750µg for *Pseudomonas aeruginosa, Xanthomonas citrovorum, Asperigillus niger* and *Asperigillus fumigatus.* Among the microbial species tested

Staphylococcus aureus was found to be highly sensitive to the ethanol extracts of fruit pulp of Graviola. The Methanol extracts of the fruit pulp of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 500µg for *Corynebacterium diphtheria* and *Staphylococcus aureus.,* while it was 750µg for *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Xanthomonas citrovorum, Proteus vulgaris, Asperigillus niger, Asperigillus fumigatus* and *Candida albicans.* Among the microbial species tested *Asperigillus niger* was found to be highly sensitive to the methanol extracts of fruit pulp of Graviola. **(Table 3: Inhibitory activity of solvent extracts of fruit pulp of Graviola or Gishta).**

The ether and ethanol extracts of seeds of Graviola or Gishta (Annona spp. of Ethiopia) shown increased zones of inhibition for the test bacteria *Corynebacterium diphtheria,* the fungal organism highly sensitive to methanol and chloroform extract was *Asperigillus niger.* The ether, chloroform and methanol extract of fruit pulp of Graviola or Gishta (Annona spp. of Ethiopia) shown increased zones of inhibition against fungal spp. The ethanol extract exhibited high activity against the growth of *Corynebacterium diphtheria*.

Table No: 4 reveals the Ether extracts of leaves of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms, the minimum inhibitory concentration was found to be 500µg for all tested microorganisms except *Asperigillus niger* and *Asperigillus fumigatus.* Among the microbial species tested *Candida albicans* was found to be highly sensitive to the ether extracts of leaves of Graviola or Gishta. The Chloroform extracts of the leaves of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 500µg for *Candida albicans,* while it was 750 µg for all the test organisms. Among the microbial species tested, *Asperigillus niger* was found to be highly sensitive to the chloroform extracts of leaves of Graviola or Gishta. The Ethanol extracts of the leaves of Graviola or Gishta are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 500µg for *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Corynebacterium diphtheria, Proteus vulgaris, Staphylococcus aureus* and *Candida albicans,* while it was 750µg for *Xanthomonas citrovorum, Asperigillus niger* and *Asperigillus fumigatus.* Among the microbial species tested *Corynebacterium diphtheria* was found to be highly sensitive to the ethanol extracts of leaves of Graviola. The Methanol extracts of the leaves of Graviola or Gishta (Annona spp. of Ethiopia) are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 500µg for *Escherichia coli, Bacillus subtilis, Corynebacterium diphtheria* and *Staphylococcus aureus;* while it was 750µg for *Pseudomonas aeruginosa, Xanthomonas citrovorum, Proteus vulgaris, Asperigillus niger, Asperigillus fumigatus* and *Candida albicans.* Among the microbial species tested *Asperigillus niger* was found to be highly sensitive to the methanol extracts of leaves of Graviola. **(Table 4: Inhibitory activity of solvent extracts of leaves of Graviola or Gishta).**

The solvent extracts of seeds, fruit pulp and leaves Graviola or Gishta (Annona spp. of Ethiopia) shown activity against all the tested microbial species. The obtained results data statistacally analyzed and tested using a full factorial Analysis of Variance (ANOVA) model.

Description about the model used in the analysis

Statistical design and analysis of experiments are scientific procedures of designing or selecting the appropriate method of conducting the experiment, collecting/measuring reliable data based on the result of each experimental trials and analyzing the collected data using statistical techniques and procedures in order to reach on a significant conclusions about the variables and treatments/factors focused by the experiment.

Among many statistical experimental designs, Factorial design became appropriate for this study, this is because Factorial experimental design is applicable for an experiment that involves more than one factor/independent variables each with at least one level/treatment. In this study the response variable is Diameter of Inhibition Zone (mm) which depends on three factor/independent variables; Leaf, Solvent Extracts and Concentration.

All combination of the levels of all the three factors (Table 5) is analyzed and tested using a full factorial Analysis of Variance (ANOVA) model. The test helped the investigator to test the presence of Main Effect of the three factors or Interaction Effect within the three factors. However ten different microorganisms are also used as a block in order to minimize the difference due to the non homogeneity of characteristics of experimental units that are Microorganisms **(Table 5: Summary of the factor variables for ANOVA model)**.

Analysis of the data

Analysis of Variance for Response, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Test Samples	2	1130.40	1130.40	565.20	43.86
Solvent Extract	3	190.93	190.93	63.64	14.94
Concentration	4	6130.30	6130.30	1532.58	118.92
Test Samples *Solvent Extract	6	1801.21	1801.21	300.20	23.30
Test Samples *Concentration	8	10186.56	10186.56	1273.32	98.81
Solvent Extract* Concentration	12	5086.84	5086.84	423.90	32.89
Test Samples *Solvent Extract* Concentration	24	2530.92	2530.92	105.45	8.18
Microorganisms	9	14435.36	14435.36	1603.93	124.46
Error	1731 22307.29 22307.29 12.89				
Total	1799 63799.80				

The result displayed in the ANOVA table revealed that there is a significant Main effect, which indicates the factor variables have a significant different effect on Diameter of Inhibition Zone (mm), and there is an interaction effect between the factors. However, the table doesn't explain which levels of the three factors are important. So to identify the effect of factor(s) level using different graphical comparisons were clearly shown below

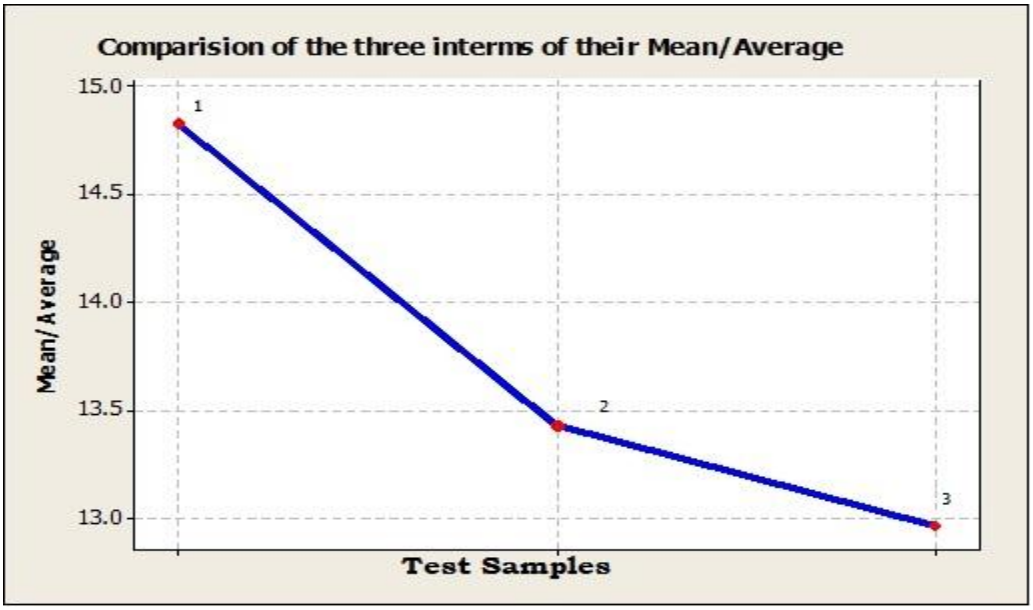
(Graph. 1: Comparision of different test samples of of Graviola (Gishita) cultivar of Ethiopia, North East Africa in terms of their antimicrobial efficacy).

As the ANOVA table proved above, Graph 1 visually depicts that the average Diameter of Inhibition Zone (mm) in Test Sample 1 is significantly larger than in Test Sample 2 and 3 but there is no significant difference between Test Sample 2 and 3.

The Graph 2 visually describes that the effect of Methanol is significantly greater than Ethanol, Chloroform and Ether. Also Chloroform is significantly greater than Ethanol but there is no statistically significant different effect between Chloroform and Ether.

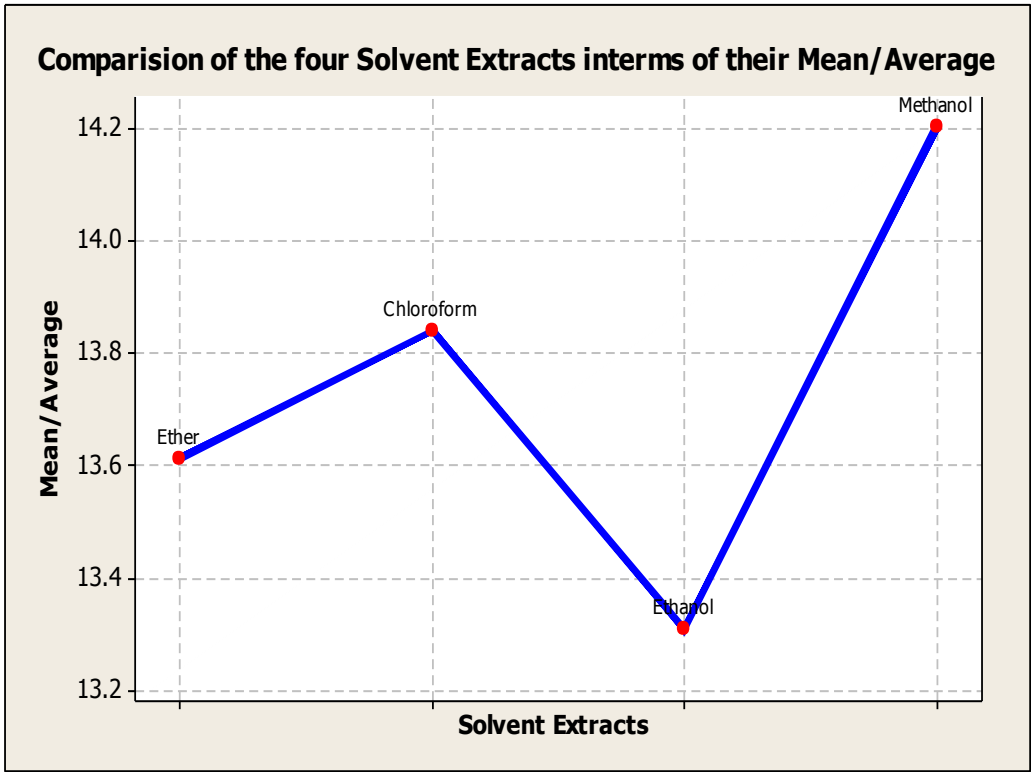
Whereas the Graph 3 shows that, as the concentration of solvent extracts increase the average Diameter of Inhibition Zone (mm) exponentially increases and this increase is statistically significant as proved in the ANOVA table. More specifically, the treatments, which are 250µg, 500µg, 750µg and 1000µg, are more effective in increasing the average diameter of Inhibition Zone (mm) compared to the control.

(Graph. 2: Comparision of efficacy of organic solvent extracts of test samples of Graviola (Gishita) cultivar of Ethiopia, North East) and (Graph. 3: Comparision of efficacy of different concentrations of the test samples of Graviola (Gishita) cultivar of Ethiopia, North East Africa).

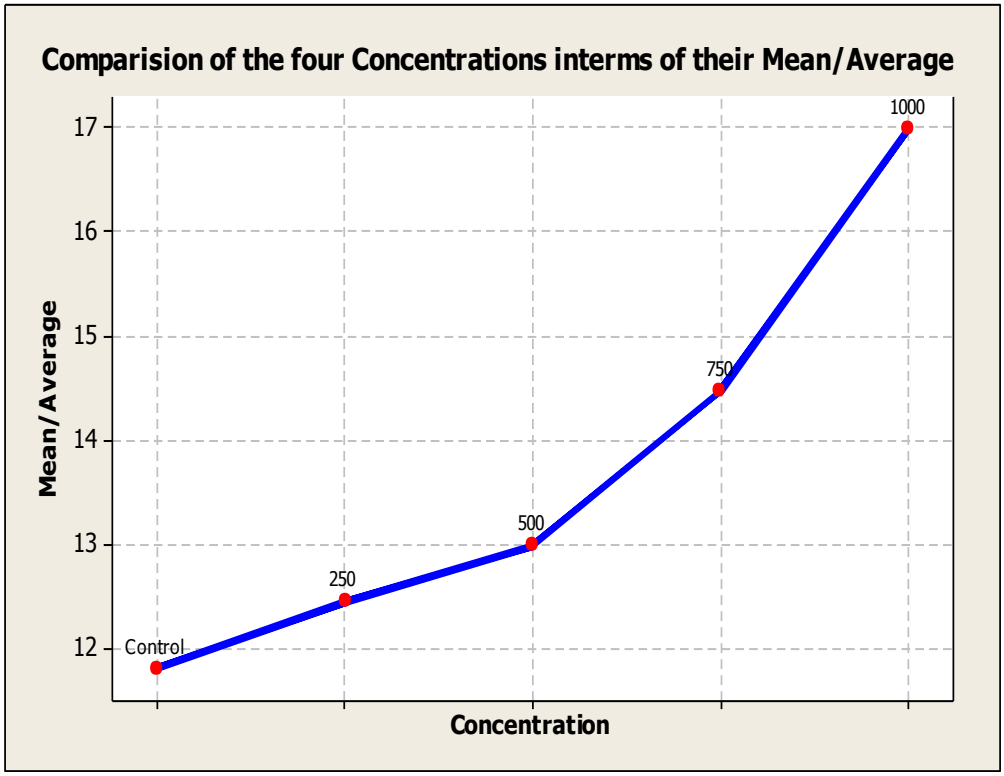


Graph. 1: Comparision of different test samples of of Graviola (Gishita) cultivar of Ethiopia, North East Africa in terms of their antimicrobial efficacy.

Test Sample 1: Seeds, Test Sample 2: Fruit pulp, Test Sample 3: Leaves



Graph. 2: Comparision of efficacy of organic solvent extracts of test samples of Graviola (Gishita) cultivar of Ethiopia, North East



Graph. 3: Comparision of efficacy of different concentrations of the test samples of Graviola (Gishita) cultivar of Ethiopia, North East Africa

Table 1: List of the selected test organisms (Bacteria and Fungi)

S.No.	Name of the organism	Characteristic features	Diseases caused by organism
1.	*Escherichia coli* (ATCC 25922)	Gram –ve rod shaped	Gastroenteritis
2.	*B. subtilis* (ATCC 6633)	Gram +ve rod shaped	Food poisoning
3.	*Pseudomonas aeruginosa* (ATCC 27853)	Gram –ve rod shaped	Wounds and Urinary tract infections
4.	*Corynebacterium diphtheria* (ATCC 75415)	Gram +ve rod shaped	Diphtheria
5.	*Xanthomonas citrovorum* (ATCC 8082)	Gram –ve rod shaped	Urinary tract infections
6.	*Proteus vulgaris* (ATCC 638)	Gram –ve rod shaped	UTI and Wound infections
7.	*Staphylococcus aureus* (ATCC 25923)	Gram +ve coccus, facultative anaerobe	Minor skin infections, Pneumonia, Meningitis, Osteomyelitis, Endocarditis, Toxic shock syndrome(TSS)
8.	*Aspergillus niger* (NCIM596)	Dichotomous branches, filamentous	Allergy, Asthma
9.	*A. Fumigatus* (NCIM 291)	Dichotomous branches, filamentous	Pulmonary haemorrhage, pneumonia.
10.	*Candida albicans* (NCIM670)	Dimorphic	Oral thrush, Gastritis, Cutaneous infections

Table 2: Inhibitory activity of solvent extracts of seeds of Graviola or Gishta

Solvent extract	Product (µg)	Zone of Inhibition (mm)									
		A	B	C	D	E	F	G	H	I	J
Ether	Control	7.00	7.00	7.00	7.00	7.00	7.00	7.00	15.60	15.60	15.60
	250	8.50	8.20	7.50	10.50	9.30	8.10	8.10	15.60	15.60	15.60
	500	13.00	13.00	7.50	23.00	19.00	8.50	8.50	15.60	15.60	18.60
	750	21.00	21.00	12.00	28.00	26.00	11.21	11.21	28.00	26.00	26.00
	1000	30.00	30.00	21.00	36.00	32.00	23.21	21.43	31.00	30.00	32.00
Chloroform	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00	15.60	15.60	15.60
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00	15.60	15.60	15.60
	500	9.00	9.00	9.00	10.00	9.00	9.00	9.00	15.60	15.60	20.00
	750	13.00	13.00	18.00	16.00	16.00	15.00	15.00	21.60	19.60	24.00
	1000	18.00	18.00	23.00	23.00	22.00	22.57	21.43	30.71	28.51	28.00
Ethanol	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00	10.00	10.00	10.00
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00	10.00	10.00	10.00
	500	11.00	11.00	9.00	11.00	9.00	10.00	10.20	10.00	10.00	12.00
	750	17.00	17.00	13.00	18.00	13.00	12.60	21.43	13.00	12.00	14.00
	1000	23.00	23.00	17.00	26.00	17.00	15.60	23.43	15.00	14.20	17.00
Methanol	Control	8.60	8.60	8.60	8.60	8.60	8.60	8.60	15.60	15.60	15.60
	250	8.60	8.60	8.60	8.60	8.60	8.60	8.60	18.60	18.60	18.60
	500	9.00	9.00	8.60	13.00	8.60	8.60	12.21	18.60	18.60	18.60
	750	12.00	12.00	9.00	17.00	9.00	12.60	21.50	22.16	20.16	22.12
	1000	16.00	16.00	13.00	24.00	14.00	12.60	38.00	39.71	36.51	31.12
Std*	10	26.03	28.55	13.73	25.81	26.00	17.00	19.08	12.66	12.66	17.00

*Standard: Streptomycin (10µg/ml) for Bacteria, Nystatin (10µg/ml) for Fungi

A) *Escherichia coli* B) *Bacillus subtilis* C) *Pseudomonas aeruginosa* D) *Corynebacterium diphtheria*
E)*Xanthomonas citrovorum* F) *Proteus vulgaris* G) *Staphylococcus aureus* H) *Asperigillus niger*
I) *Asperigillus fumigatus* J) *Candida albicans*

Table 3: Inhibitory activity of solvent extracts of fruit pulp of Graviola or Gishta

Solvent extract	Product (µg)	Zone of Inhibition (mm)									
		A	B	C	D	E	F	G	H	I	J
Ether	Control	7.00	7.00	7.00	7.00	7.00	7.00	7.00	15.60	15.60	15.60
	250	8.05	7.84	7.35	9.45	8.61	7.77	7.77	15.60	15.60	15.60
	500	11.20	11.20	7.35	18.20	15.40	8.05	8.05	15.60	15.60	17.70
	750	16.80	16.80	10.50	21.70	20.30	9.94	9.94	24.28	22.88	22.88
	1000	23.10	23.10	16.80	27.30	24.50	18.34	17.10	26.38	25.68	27.08
Chloroform	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00	15.60	15.60	15.60
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00	15.60	15.60	15.60
	500	9.00	9.00	9.00	9.00	9.00	9.00	9.00	15.60	15.60	18.68
	750	11.80	11.80	15.30	13.90	13.90	13.20	13.20	19.80	18.40	21.48
	1000	15.30	15.30	18.80	18.80	18.10	18.49	17.70	26.17	24.63	24.28
Ethanol	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00	10.00	10.00	10.00
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00	10.00	10.00	10.00
	500	10.40	10.40	9.00	10.40	9.00	9.70	9.84	10.00	10.00	11.40
	750	14.60	14.60	11.80	15.30	11.80	11.52	17.70	12.10	11.40	12.80
	1000	15.86	15.86	12.92	17.33	12.92	13.62	19.10	13.50	12.94	14.90
Methanol	Control	8.60	8.60	8.60	8.60	8.60	8.60	8.60	15.60	15.60	15.60
	250	8.60	8.60	8.60	8.60	8.60	8.60	8.60	17.70	17.70	17.70
	500	8.80	8.80	8.60	11.68	8.60	8.60	11.13	17.70	17.70	17.70
	750	10.98	10.98	8.88	14.48	8.88	11.40	17.63	20.19	18.79	20.16
	1000	13.78	13.78	11.68	19.38	12.38	11.40	29.18	32.47	30.23	26.46
Std*	10	26.03	28.55	13.73	25.81	26.00	17.00	19.08	12.66	12.66	17.00

*Standard: Streptomycin (10µg/ml) for Bacteria, Nystatin (10µg/ml) for Fungi

A) *Escherichia coli* B) *Bacillus subtilis* C) *Pseudomonas aeruginosa* D) *Corynebacterium diphtheria* E)*Xanthomonas citrovorum* F) *Proteus vulgaris* G) *Staphylococcus aureus* H) *Asperigillus niger* I) *Asperigillus fumigatus* J) *Candida albicans*

Table 4: Inhibitory activity of solvent extracts of leaves of Graviola or Gishta.

Solvent extract	Product (µg)	Zone of Inhibition (mm)									
		A	B	C	D	E	F	G	H	I	J
Ether	Control	7.00	7.00	7.00	7.00	7.00	7.00	7.00	15.60	15.60	15.60
	250	7.90	7.72	7.30	9.10	8.38	7.66	7.66	15.60	15.60	15.60
	500	10.6	10.6	7.30	16.6	14.2	7.90	7.90	15.60	15.60	17.40
	750	15.4	15.4	10.00	19.6	18.4	9.52	9.52	23.04	21.84	21.84
	1000	20.8	20.8	15.4	24.4	22.00	16.72	15.65	24.84	24.24	25.44
Chloroform	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00	15.60	15.60	15.60
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00	15.60	15.60	15.60
	500	9.00	9.00	9.00	10.00	9.00	9.00	9.00	15.60	15.60	20.00
	750	11.4	11.4	14.4	13.2	13.2	12.6	12.6	19.20	18.00	20.64
	1000	14.4	14.4	17.4	17.4	16.8	17.14	16.45	24.66	23.34	23.04
Ethanol	Control	9.00	9.00	9.00	9.00	9.00	9.00	9.00	10.00	10.00	10.00
	250	9.00	9.00	9.00	9.00	9.00	9.00	9.00	10.00	10.00	10.00
	500	10.2	10.2	9.00	10.2	9.00	9.60	9.72	10.00	10.00	11.20
	750	13.8	13.8	11.4	14.4	11.4	10.8	16.45	11.80	11.2	12.40
	1000	17.4	17.4	13.80	19.20	13.80	12.96	17.65	13.00	12.52	14.20
Methanol	Control	8.60	8.60	8.60	8.60	8.60	8.60	8.60	15.60	15.60	15.60
	250	8.60	8.60	8.60	8.60	8.60	8.60	8.60	17.40	17.40	17.40
	500	8.84	8.84	8.60	11.24	8.60	8.60	10.76	17.40	17.40	17.40
	750	10.64	10.64	8.84	13.64	8.84	11.00	16.34	19.53	18.33	19.51
	1000	13.04	13.04	11.24	17.84	11.84	11.00	26.24	30.06	28.14	24.91
Std*	10	26.03	28.55	13.73	25.81	26.00	17.00	19.08	12.66	12.66	17.00

*Standard: Streptomycin (10µg/ml) for Bacteria, Nystatin (10µg/ml) for Fungi

A) *Escherichia coli* B) *Bacillus subtilis* C) *Pseudomonas aeruginosa* D) *Corynebacterium diphtheria* E)*Xanthomonas citrovorum* F) *Proteus vulgaris* G) *Staphylococcus aureus* H) *Asperigillus niger* I) *Asperigillus fumigatus* J) *Candida albicans*

Table 5: Summary of the factor variables for ANOVA model

Factor	Type	Number of Levels	Values
Test Samples	fixed	3	Leaves , Fruit pulp, Seeds
Solvent Extract	fixed	4	Ether, Chloroform, Ethanol, Methanol
Concentration (µg/ml)	fixed	5	Control, 250, 500, 750, 1000
Microorganisms	Fixed/Block	10	A) *Escherichia coli* B) *Bacillus subtilis* C) *Pseudomonas aeruginosa* D) *Corynebacterium diphtheria* E)*Xanthomonas citrovorum* F) *Proteus vulgaris* G) *Staphylococcus aureus* H) *Asperigillus niger* I) *Asperigillus fumigatus* J) *Candida albicans*

DISCUSSION

Among different solvent extracts of Graviola or Gishta (Annona spp. of Ethiopia), all the solvent extracts are inhibitory to all the test organisms, but the seeds extracts shown high activity against tested microorganisms followed by fruit pulp and leaves extracts. The ether and ethanol extracts of seeds of Graviola or Gishta (Annona spp. of Ethiopia) shown increased zones of inhibition for the test bacteria *Corynebacterium diphtheria,* the fungal organism highly sensitive to methanol and chloroform extract was *Asperigillus niger.* The ether, chloroform and methanol extract of fruit pulp and leaves of Graviola or Gishta (Annona spp. of Ethiopia) shown increased zones of inhibition against fungal spp. The ethanol extract exhibited high activity against the growth of *Corynebacterium diphtheria*. The inhibitory activities of different solvent extracts were enhanced with increase in concentration. Literature revealed that various species of Annona claimed their medicinal importance and has been scientifically proven for their potent antimicrobial and biological activities[4].

IV. CONCLUSION

Phytomedicines are effective in treating most of the infectious diseases mainly skin infections. Most of the secondary metabolites, serve as a plant defence mechanism against microorganisms, insects and herbivores[9]. Recent studies have supported many of soursop's traditional medicinal uses and also showed that various parts of the tree contains potential bioactive compounds, which have been shown to be responsible for its myriad array of its medicinal attributes. Acetogenins exhibited a broad range of potent biological activities[10]. The results suggest that extracts are effective against the tested organisms. The values reveal the medicinal properties of the experimental plant, some extracts are found better than standard values. The obtained result in the present work may also provide a support to the uses of the plant in the traditional medicine. Further work is needed to isolate the active principle from the plant extracts and to carry out pharmaceutical studies

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VI. CONFLICT OF INTEREST

Conflict of interest declared none.

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