REVIEW ARTICLE

A REVIEW ON ANTIBACTERIAL PROPERTIES OF EXTRACTS FROM PSIDIUM SPP AND EFFECT OF THE EXTRACTION SOLVENT

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ABSTRACT

The vast potential of plants has bioactive compounds that could be effective or inhibitory to microorganisms. Several experiments aimed at understanding the plant composition and its safe usage in the modern world have been conducteddue to their traditional importance in herbal medicine. Psidiumsppis a phyto-therapeutic plant believed to have active components that helps to manage and/ or treat different disease conditions such as vomiting, fever, diarrhea, dysentery, ulcer etc. Thus, understanding of the antimicrobial nature via research on their extracts will further explain their role in the history of herbal medicine and application in modern world.After application of inclusion and exclusion criteria, a systematic review composed of sixteen published research articles of twenty trials from different parts of Psidiumspp extract against Escherichia coli and Staphylococcus aureus were appraised. The outcome was evaluated via zones of inhibition with consideration to the extraction solvent and the plant part. Analysis of the available data showed that the choice of solvent (95% C.I) affected the amount of composition extracted in the order of methanol, aqueous, acetone and ethanol while the plants part also varied in terms of their bioactive properties to inhibit the target organism in order of leaf, fruit, stem bark, twig and seed. Due to the ability of these extracts to inhibit the target organisms, it can therefore be deduced that concentration of the active components of Psidiumspp can be used as an alternative to treat diseases related to E. coli and S.aureus.

KEY WORDS: Bioactive, Psidiumspp, Escherichia coli, Staphylococcus aureus, Antibiotics.

INTRODUCTION

Most of the major health problems faced by developing countries are caused by infectious microorganisms that have developed resistance to a number of available antibiotics thus leading to difficulties in treatment[1] of the disease.

Generally, before the advent of modern drugs which took its root from traditional medicine, nature (plants) has always been significant in the treatment of diseases and their conditions such as dysentery, diarrhea, vomiting, wounds, sore throat etc[2].

Intensive studies in to natural therapy in the past decades till date, as further proven plants to be a valuable natural product which can help in the maintenance of human health and this is due to their varieties of bioactive components[3] (tannins, flavonoids, cellulose, etc.). These plants are believed to be therapeutic due to their chemical constituents[4] and thus several published research have been aimed to investigate, unlock and further understand the properties and effects of plant extracts. These properties such as anti-inflammatory, anti-fungal, antibacterial, anti-cough, anti-depressant, hypoglycemic, anti-mutagenic, antispasmodic and anti-diarrhea have been investigated with different outcomes or results both at traditional and modern level[5, 6, 7, 8]. In the production of new drugs, the plant secondary metabolites have been found to be a source of phytochemicals to be used as an intermediate[9].

The term guava which appears to be derived from a Spanish word guayaba is a fruit native to Mexico and America (Central, Northern and Southern) [3]. It is

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a common tropical fruit from the family of Myrtaceae and botanically referred to as Psidium spp[10]. It comprises of about 133 genera and 3800 species with Psidiumguajava (apple, guava), Psidiumlittoralevarlittoral(lemon, guava), Psidiumlittoralevarcattlelanum(strawberry guava) and Psidiumguajava L. (pink guava) been the most common specie[11]. They are characterized by simple, elliptic to ovate tough, dark leaves of 5 to15cm long. They have many seeded berries as fruit and white colored flowers consisting of five petals and numerous stamens (http://www.rain-tree.com/guava.htm: NC 27401-4901 USA).

Considering the vast potentiality of plants as sources for antimicrobial drugs, a systematic review which can be referred to as the use of plain and systematic methods in answering questions of specific interests[12] is needed to correlate the investigations for further research and intervention interests. This involves the identification, selection and appraisal of relevant research articles for data collection and analysis[12, 13]. It is usually rigorously and transparently done to prevent bias and produce credible evidence based results[14]. Furthermore, a systematic review helps to identify discrepancies in studies and promotes precision which gives decision maker's power to make choices[13].

This method was adopted to synthesize findings from different primary studies rather than relying on the evidence of a single study[15] to evaluate Psidiumspp as an effective, bioactive source with antibacterial activity against common representative members of gram positive (Staphylococcus aureus) and negative (Escherichia coli) isolate, standard strains and/or known resistance strains. This was done with respect to their extraction solvent and also to evaluate from published articles the potential applicability of its components in the treatment of infectious disease.

METHODS

This is a systematic review that adopts the framework of Population, Intervention, Comparison and Outcome (PICO) by Bettany-Saltikov[16] to identify, evaluate, and combine quality evidence of scientific standard that meets specific criteria in order to answer the question of interest[13]. It is a transparently carried out study to prevent bias and produce credible evidence based results[14] which promotes precisions and identifies discrepancies[17].

Research Strategies

The framework helps the researcher to access the full range of the available literatures that addresses the research question while creating direction for the literature search[18]. It involves four fragments which are summarized as PICO;

P: Psidiumspp (Guava), I: Extracts (Leaf, Fruit and Bark) and extract solvent (methanol, acetone, ethanol and aqueous), C: Antimicrobial activity –Antibacterial (Inhibition zone) and O: Effect of solvent, Outcome, Part with potential.

Inclusion And Exclusion Criteria

The selected articles (16) included double-blind, randomized, placebo-controlled trials that access the extract of Psidium different parts as an antimicrobial agent to pathogenic microorganisms from 2006 to 2016. Only a trial involving one or both of S. aureus and E. coli with respect to zone of inhibition by the Psidiumspp specific extract. Evaluated data also included results from laboratory or in-vitro studies and trialsrespective of extraction method used.

The excluded articles were studies not within the selected year of interest, studies without extracts from Psidiumspp and studies with combined extracts from other source without clear differentiation. Studies without at least one of the target solvent were excluded, studies not presented in the English language and those with incomplete information were also excluded.

Types Of Intervention

This review is targeted to examine Psidiumspp as an alternative antibiotic to S. aureus and E. coli and elucidate role of extraction solvent used

Outcome Measures

The measured primary outcomes were antibacterial (zone of inhibition), the effect of the solvent and the Psidium extract with most bio active potential.

Search Criteria

The adapted method used to conduct the systematic search for all relevant literature was the PICO formula of population/problem, intervention, comparison, and outcome. The online search queries were refined using the subject headings while constantly interchanging keywords such as: Guava, extracts, antibacterial, antifungal, antiviral, effect of guava extracts, medicinal parts of guava etc. The studies were obtained from computerized searches of multiple electronic bibliographic databases.

Quality Assessment Of Studies

Studies were assessed for quality using standard tools; Quantitative Education Tool (via assessment of methodology and outcome) and components approach by multiple reviewers. Analysis on an intention-to-treat basis was also assessed. Critical analysis (critique of hypothesis, methods, results and conclusion) was done to ensure studies are of standard quality and the results can be generalized.

Data Collection

Information and data were extracted from each study that met the inclusion criteria so that

evidence can be evaluated, presented, and summarized.

Data Analysis

The analysiswas done manually and important data of the summary and comparisons were presented in table and figure.

RESULTS

This section is targeted to critically examine the quality of methodology used in the included articles and compare the results with other studies to ensure validity and reliability

Quantity And Quality Of Study Evidence

Search results from Google scholar, PubMed, Medline provided 6290 publications were 150 with potential relevance remained after the removal of duplicates and irrelevant studies. Application of the inclusion and exclusion criteria resulted in to 16 published research articles.These articles presented 24 trials that have been summarized in Table1.Transparency in methodology with the stated source of the target plant and organism were made available by all authors of selected articles except one i.e. Malaviya and Mishra[19]. Only Balangcodet.al;[20] made provision for approval before plant material collections while the deposition of voucher was made known by Joseph and Priya[21], Anas et.al;[22] and Thiyagarajanand Jamal[23].

Confirmation of target (clinical) isolates by conventional procedures and provision of required information for standard strains (non-isolate) were made available by studies from Omoregie et.al;[24], Bansoda and Chavan[7], Joseph and Priya[21], Nath and Bhattacharjee[25], Esimone et.al;[26],Balangcod et.al;[20], Anas et.al;[22] and Tauraet.al;[27] while information by Malaviya and Mishra[16] was not clearly stated. Ismail et.al;[28], Tahera et.al;[29], Mushtaq et.al;[30], Ali et.al;[31], Balangcod et.al;[20], Romasi et.al;[32] and Thiyagarajanand Jamal[23] failed to provide at all. A study by Taura et.al;[27] claim to have done confirmation of target organism, but not to the level of space for all the organisms.

Results presented by Ismail et.al;[28], Joseph and Priya[21], Mushtaq et.al;[30], Zahidah et.al;[33] and Thiyagarajan and Jamal[23] added strength to their studies by clearly stating the use of statistical tools such as SPSS (Statistical Package for the Social Science), ANOVA (Analysis of Variance), Graph-pad prism and SAS (Statistical Analysis System) to generate p values, standard deviation and mean which reflected in their results and thereby increasesthe reliability, however, use of basic mean from triplicate result was noticed in all studies.

Phytochemical analysis of extracts to expose their composition and further indicate active components was done except for studies from Ismail et.al;[28], Joseph and Priya[21], Nathand Bhattacharjee[25], Tahera et.al;[29], Malaviya and Mishra[19], Mushtaq et.al;[30], Ali et.al;[31] and Anas et.al;[22]

The use of control (known antibiotics i.e. Sparfloxacin, Erythromycin, Flouconazole, Ampicillin, Tetracycline, Kanamycin, Streptomycin,Vancomycin, Chloramphenicol, Penicillin G, Cloxacillinetc) to further strengthen the trials and the displays of activity index of the extracts on target organisms were included in studies from Omoregie et.al;[24], Bansoda and Chavan[7], Nath and Bhattacharjee[25], Mushtaq et.al;[30], Balangcod et.al;[20], Zahidah et.al;[33], Anas et.al;[22], Taura et.al;[27] and Thiyagarajan and Jamal[23]. Study by Esimone et.al;[26] on methicillin-resistance S.aureus which used oxacillin as control for resistance and the strains were classified as resistant according to CLSI[34] guidelines. The studies by Joseph and Priya[21] and Tahera et.al;[29] made reference to controls by ampicillin and gentamycin/ streptomycin respectively however, no presented result was found.

A total of twenty-four trials from sixteen articles of original research that met the inclusion criteria were all summarized in Table 1. Figure 1 displays the total inhibition activity of all extract from the selected articles in respect to the target organisms while Figure 2 and 3 shows the generated activity index of extraction solvents for S. aureus and E. coli.

Table 1: Summary of results from selected article

		Staphylococcusaureus				Escherichia coli			
		ZONE OF INHIBITION (mm) from different extraction							
REFERENCE	PLANT PART	M	AC	E	AQ	M	AC	E	AQ
Ismailet.al; 2012	Leaf	19.00	-	-	20.00	16.70	-	-	20.00
Omoregie et.al; 2010	Leaf	30.00	-	24.00	-	30.00	-	00.00	-
		32.00	-	29.00	-	30.00	-	00.00	-
Bansode and Chavan, 2014	Leaf	-	-	-	-	05.00	04.00	03.00	-
Joseph and Priya, 2010	Leaf	15.00	18.00	-	-	-	-	-	-
Nath and Bhattacharjee 2015	Leaf & Twig	08.00	10.00	-	10.00	05.00	06.00	-	05.00
Taheraet.al; 2014	Fruit (skin+core)	00.00	-	09.21	09.00	09.13	-	00.00	10.00
		07.50	-	09.25	07.50	00.00	-	09.97	00.00
Malaviya and Mishra, 2011	Fruit	-	-	00.00	11.00	-	-	05.00	10.00
Esimoneet.al;2012	Stem bark	12.00	-	-	10.00	-	-	-	-
Mushtaqet.al; 2014	Leaf	14.50	16.00	-	-	-	-	-	-
Aliet.al; 2014	Leaf	02.00	-	-	01.00[a]	00.00	-	-	00.00[a]
					01.50[b]				00.00[b]
Balangcod et.al;2012	Leaf	13.00	-	-	-	14.00	-	-	-
ZahidahSeed et.al; 2013	Leaf	-	-	-	00.00	-	-	-	00.00
		-	-	-	10.50	-	-	-	00.00
Anaset.al; 2007	Leaf	14.00	15.00	-	15.00	-	-	-	-
		14.00	14.00	-	12.00	-	-	-	-
		21.00	20.00	-	13.00	-	-	-	-
		14.00	15.00	-	13.00	-	-	-	-
Romasiet.al; 2006	Leaf	-	-	-	00.00	-	-	-	00.00
Tauraet.al; 2014	Leaf	-	-	10.00	-	-	-	00.00	-
Thiyagarajan AndJamal,et.al; 2015	Leaf	-	-	-	-	25.00	-	20.00	13.00[b]
	-	-	-	-	27.00	-	22.00	15.00[b]	

M= Methanol, AC= Acetone, E= Ethanol, AQ= Aqueous
[a]= cold water, [b]=hot water, - = not evaluated

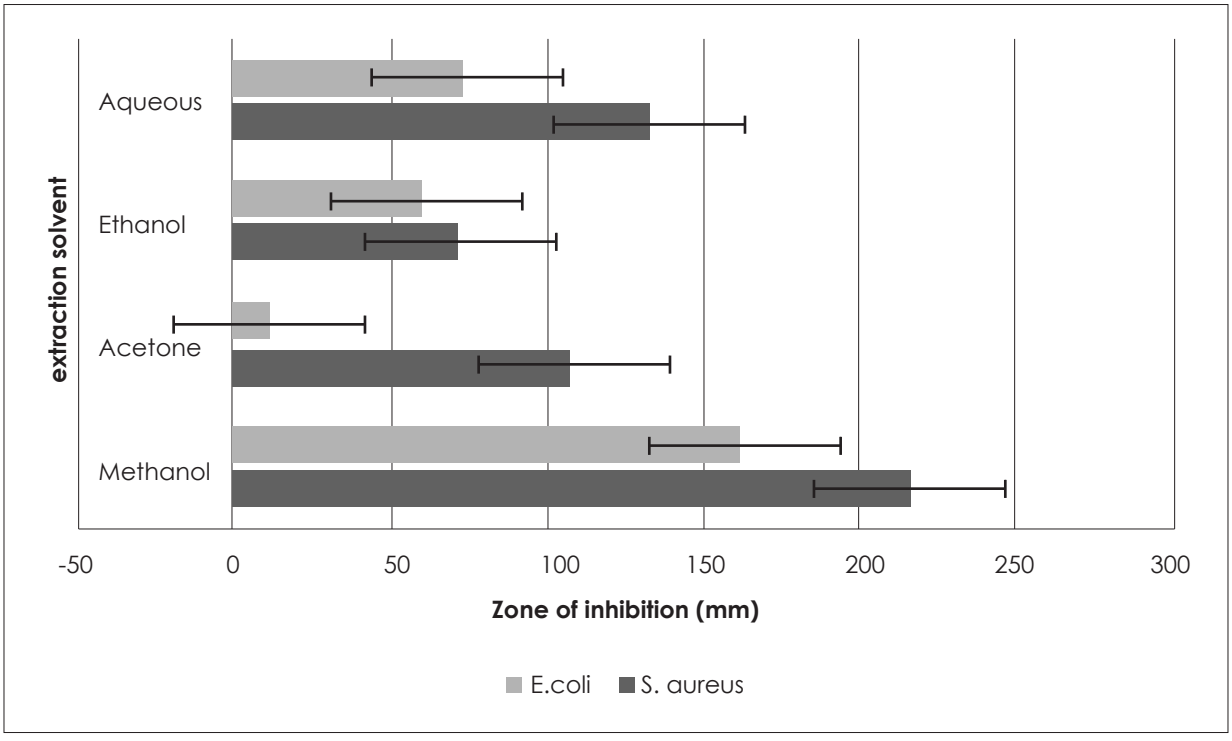


Figure 1: Total inhibition activity of all extraction solvent against test organisms

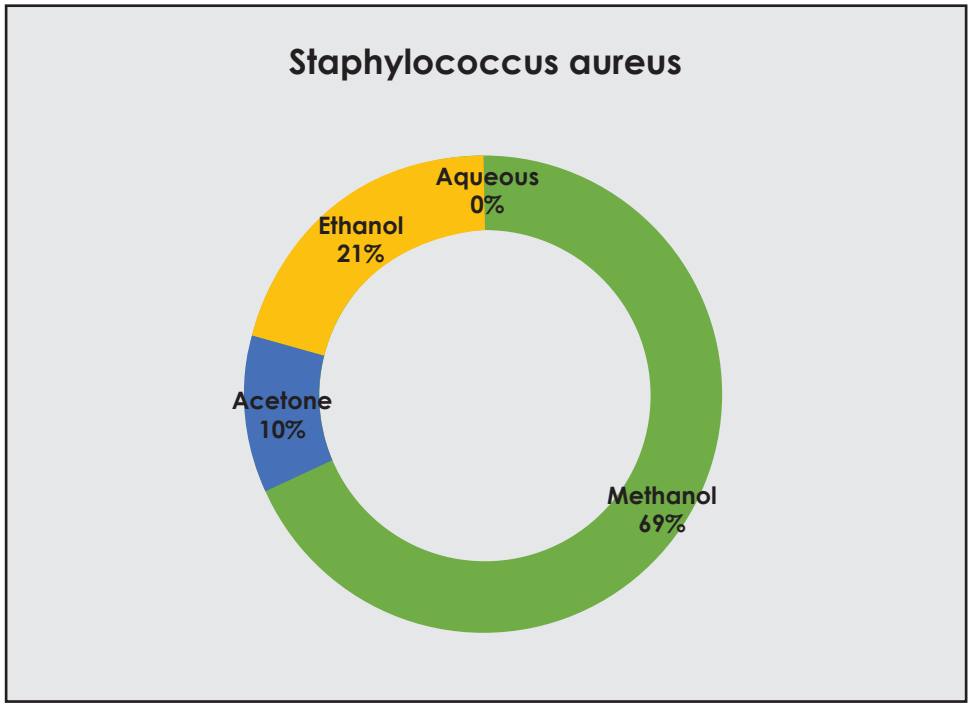


Figure 2: Activity index od extraction solvent for S.aureus

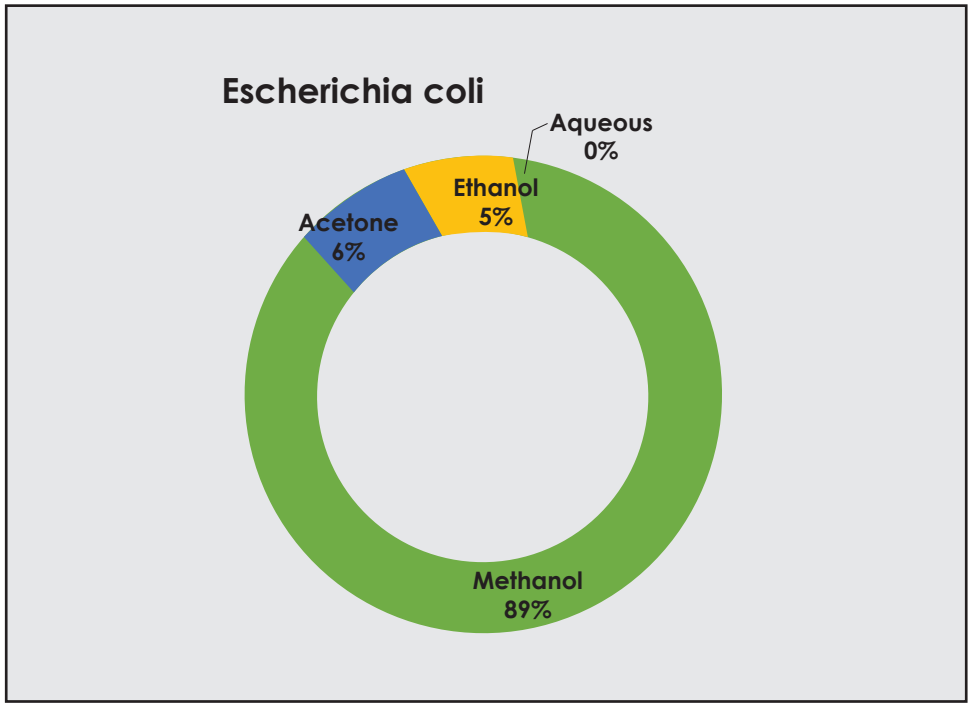
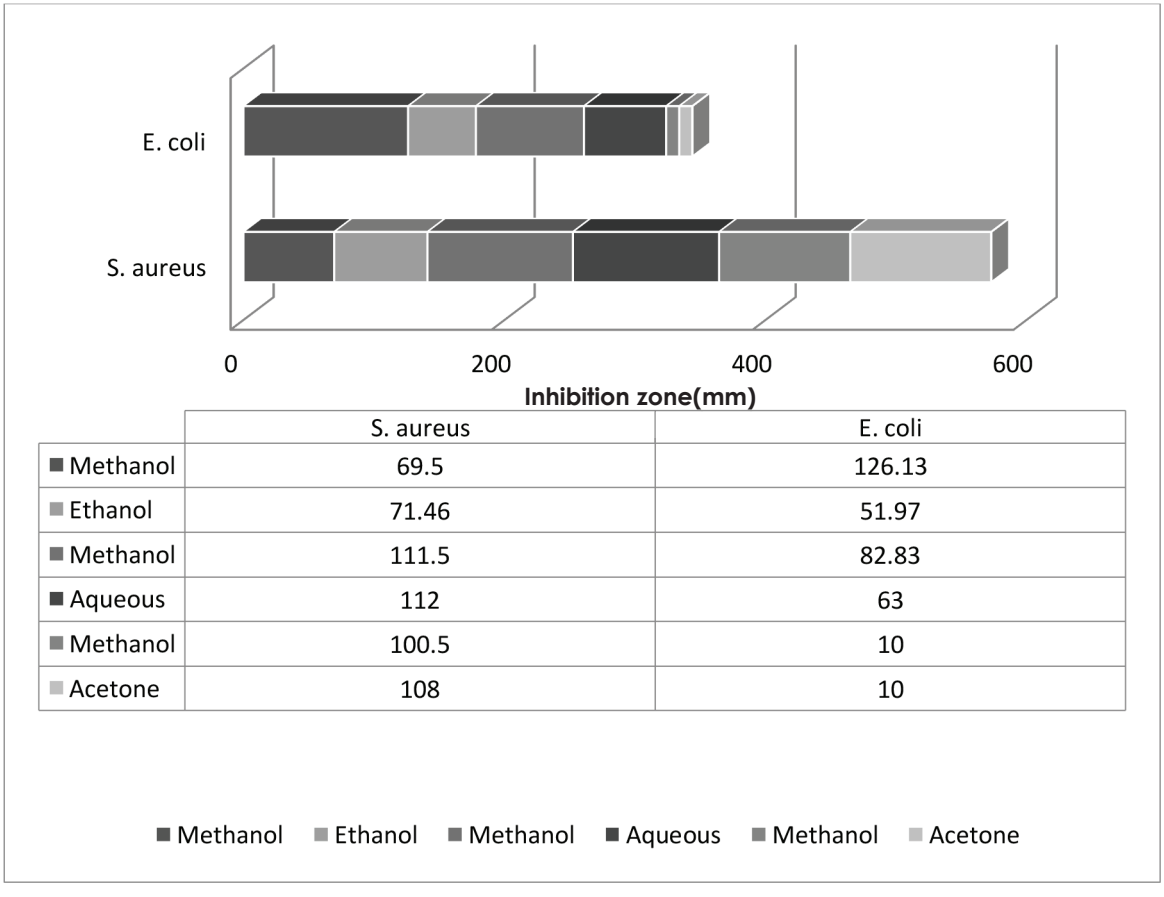


Figure 3: Activity index of extraction solvent for E. coli



	S. aureus	E. coli
Methanol	69.5	126.13
Ethanol	71.46	51.97
Methanol	111.5	82.83
Aqueous	112	63
Methanol	100.5	10
Acetone	108	10

Figure 4: Comparison of inhibition zone by different solvent

DISCUSSION

The inhibition zones corresponding to the antibacterial (antimicrobial) activity against S. aureus and E. coli were found to increase extensively with the source of the extract and the choice of solvent for the extraction. This statement is in line with that by Nisha et.al;.[35]which concluded that the quantity of oil extract plays an important role in the effect of inhibition. From comparison of the total inhibition activity based on the extraction solvent, it was noticed that methanol showed the highest number of inhibition activity (zone) to both target organism {i.e. E. coli (approx. 220mm) and S. aureus (approx-.160mm) are most sensitive to Psidium spp extract with methanol as the extraction solvent}. Extraction via aqueous solvent inhibited the growth of S. aureus more than E. coli and while extraction by acetone had the least inhibition activity against E.coliwhen compared with S. aureus and result from other solvents. These could be due to the absence/ insufficient presence of the bioactive compounds that are inhibitory to E. coli as a result of the choice of extraction solvent as compared to extraction by methanol which showed higher inhibition activity. Additionally, from fig 4, it was noticed that irrespective of the extraction solvent, E. coli (344mm) was generally more resistant to extract of Psidium spp than S. aureus (568mm) which is more sensitive. This can be further understood by examination of the phytochemical analysis of the different parts of the plant.

The Parts Of Psidium Spp

The leaf, fruit, seed, stem-bark and twig of Psidium spp has been used in the selected articles while the most commonly used one which also happens to have yielded the highest result in term of inhibition zone is the extract from the leaf part. This might be due to its easily accessible nature, choice of researcher and / or its phytochemical composition. Further comparison of the outcome with extract from leaf to other plant part e.g. the seed which was studied by Zahidah et.al;.[33] in a research to reveal the antioxidant and antimicrobial activities of pink guava leaves and seeds, it was stated that the pink guava leaf is a superior bioactive ingredient than its seed; this was also supported by its antimicrobial activity against S. aureus when the seed had none. Although there was no recorded inhibition for E. coli by product from both extracts. In addition, the phytochemical analysis specifically the total phenolic and flavonoid contents revealed a higher value from the leaf and this is similar to the result from other researchers such as Ojan and Nihorimbere[36], Wojdylo et.al;[37]

The result from the analysis of the antibacterial property of guava fruit by Tahera et.al;[29] on skin and core and that by Malaviya and Mishra[19] on the fruit shows that the fruit part of the plant holds a promising antibacterial property against both test organisms on different extraction solvents.

The leaf: Analysis by Omoregie et.al;[24], Bansoda and Chavan[7], Balangcod et.al;.[20], Romasi et.al;[32], Taura et.al;[27] and Thiyagarajan and Jamal[23] revealed the presence of Carbohydrate, Tannins, Glycosides, Saponins, Terpenes (Terpenoid) Sterols, Flavonoids, Resins, Balsams, Alkaloids, Phenolic compounds, Anthra-quinones, Triterpenoid, reducing sugar. Based on these components, analysis of the inhibitory activities from the Table 1 shows that E.coli is resistant to extraction by ethanol and aqueous (cold) but sensitive to extraction by methanol, aqueous (hot) and acetone while S. aureus is sensitive to extraction by methanol and ethanol but not aqueous.The results of the components are in line to those by Zakaria et.al;[38],Iwu[39], Nadkarni and Nadkarni[30]; Oliver-Bever[41]; Begum et.al;[42]; [43]; Wyk et.al;[44], Ghosh et.al;[45], Chen et.al;[46] and Matwally et.al;[5].

However, Romasi et.al;[32] recorded no trace of Triterpenoid when water was used as extraction solvent with no sensitivity from both organisms i.e. no zone of inhibition.Bansode and Chavan[7] also had no result for Terpenoid (methanol), Tannins and Saponins under extraction by use of ethanol but recorded a low sensitivity from E. coli. Alkaloids were also reported missing in research by Thiyagarajan and Jamal[23] with ethanol and Omoregie et.al;[24] by methanol extraction.

The Fruit and its essential oil has been indicated to be composed of Vitamin C, vitamin A, iron, calcium, Manganese, Phosphoric, Oxalic and Malic acids, Saponins combined with Oleanolic acid, flavonoids, guaijavarin, Quercetin, hexanal, -2-hexenal , 2,4-hexadienal, 3-hexenal, 2-hexenal, 3-hexenyl acetate and phenol, while β-caryophyllene, nerolidole.t.c. (Nadkarni and Nadkarni[40]; Paniandy et.al;[47], Joseph and Priya[11]) however, no phytochemical analysis was carried out by Tahera et.al;[29]and Malaviya and Mishra[19]. S. aureus showed higher sensitivity to extract from the fruit (skin and core) of Psidiumspp than E.coli. Aqueous extraction had the highest zone of inhibition for both organisms when compared to methanol and ethanol while extraction via ethanol had the least activity for both organisms.

Phenolic compound and flavonoids were reported present in the seed by Zahidah et.al;[33] and this is in line with reports by Mitchel et.al;[48]: Ojan and Nihorimbere[36] which also found Proteins, starch, oils, glycoside, quercetin-3-O-β-D-(2''-Ogalloylglucoside)-4'-O-vinylpropionate to be inclusive. The target organisms (E. coli and S. aureus) were resistant to this extract and could be due to composition of the seed or the extraction solvent.

The stem bark was found to have inhibitory activity to both target organisms with methanol and aque-

ous as the extraction solvent. The stem bark is composed of a high to moderate concentration of carbohydrates, tannins, cardiac glycosides, proteins, alkaloids, reducing sugar,saponins and oil with low concentration of steroids and terpenoids (Esimone et.al;[28]). Similar composition was earlier reported by Begum et.al;[42] and Nadkarni and Nadkarni[40]. It can thus be generalized that Psidium spp is an essential source for Tannins, Saponins, reducing sugar and glycosides.

The summary from the combination of resulting activity index based on the solvent of extraction for both organisms represented on figure 2 and 3 indicated Methanol as the best choice for extraction. It is however of important note that this deduction was made only from trials that presented data for control using standard antibiotics. Generally, important factors such as polarity, low boiling temperature to allow for easy removal of the solvent from the product, ability to not react or chemically alter the extract, low viscosity and stability to light, heat and oxygen[59] has been indicated to contribute to choice of solvent. The increase in the use of methanol could be due to its polarity index (ampiphillic), self-preservative nature, higher safety level, low boiling point (www.metanex.com/methanol/techsafetydata.html) etc.

However, the data presented on fig 4 which compares the inhibition zones from trials that utilized at least two of the solvent with respect to methanol which had the highest activity index showed that the zones of inhibition from using ethanol, acetone and aqueous were slightly higher than methanol for Staphylococcus aureus while methanol proves effective for Escherichia coli.

Conclusively, it can be deduced that Psidium spp is a significant source of bioactive agents (compounds) and thus amongst other medicinal plants, it can be used to minimize the ever increasing incidence of antibiotic resistant microorganisms. Different parts of the plant have been reported to cure one form of disease or the other in herbal usage however; there is need for clinical trials in support of product development and modern approach of usage for target effectiveness. These will help to avoid unnecessary complications such as over dosage and side effect that are common to most local herbal prescriptions.Use of methanol and Aqueous as solvent proved most successful of all followed by ethanol and acetone and thus it is advised that interested researchers should use the best of them for effective extraction of plant components.

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