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 conducted due to their traditional importance in herbal medicine. Psidium spp is 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 Psidium spp 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 Psidium spp can be used as an alternative to treat diseases related to E. coli and S. aureus.

KEY WORDS: Bioactive, Psidium spp, 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 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.

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 is due to their varieties of bioactive components (tannins, flavonoids, cellulose, etc.). These plants are believed to be therapeutic due to their chemical constituents 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, anti-spasmodic and anti-diarrhea have been investigated with different outcomes or results both at traditional and modern level. 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.

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). It is

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a common tropical fruit from the family of Myrtae-
ae and botanically referred to as Psidium spp\textsuperscript{10}. It
comprises of about 133 genera and 3800 species
with Psidiumguajava (apple, guava), Psidiumlittorale-
levator (lemon, guava), Psidiumlittorallevarcat-
tielanan (strawberry guava) and Psidiumguajava L.
(pink guava) been the most common specie\textsuperscript{11}.
They are characterized by simple, elliptic to ovate
flattened, dark leaves of 5 to 15 cm 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 sour-
ces 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\textsuperscript{12,13} is needed to correlate the investiga-
tions for further research and intervention
interests. This involves the identification, selection
and appraisal of relevant research articles for data
collection and analysis\textsuperscript{12,13}. It is usually rigorously and
transparently done to prevent bias and produce
credible evidence based results\textsuperscript{14}. Furthermore, a
systematic review helps to identify discrepancies in
studies and promotes precision which gives decision
maker's power to make choices\textsuperscript{13}.

This method was adopted to synthesize findings
from different primary studies rather than relying on
the evidence of a single study\textsuperscript{15} to evaluate Psidium-
spp as an effective, bioactive source with antibac-
terial activity against common representative mem-
bers 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 evalu-
ate from published articles the potential applicabil-
ity of its components in the treatment of infectious
disease.

METHODS

This is a systematic review that adopts the frame-
work of Population, Intervention, Comparison and
Outcome (PICO) by Bettany-Saltikov\textsuperscript{16} to identify,
evaluate, and combine quality evidence of scientif-
ic standard that meets specific criteria in order to
answer the question of interest\textsuperscript{13}. It is a transparently
carried out study to prevent bias and produce
credible evidence based results\textsuperscript{14} which promotes
precisions and identifies discrepancies\textsuperscript{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\textsuperscript{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 anti-
microbial 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 trials respective 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 differentia-
tion. 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 system-
atic search for all relevant literature was the PICO
formula of population/problem, intervention, com-
parison, 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 general-
ized.

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 analysis was 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 Table 1. 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. Only Balangcod et al. made provision for approval before plant material collections while the deposition of voucher was made known by Joseph and Priya, Anas et al. and Thiyagarajan and Jamal.

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., Bansoda and Chavan, Joseph and Priya, Nath and Bhattacharjee, Esmone et al., Balangcod et al., Anas et al. and Taura et al., while information by Malaviya and Mishra was not clearly stated. Ismail et al., Tahera et al., Mushhaq et al., Ali et al., Balangcod et al., Romasi et al., and Thiyagarajan and Jamal failed to provide at all. A study by Taura et al. 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., Joseph and Priya, Mushhaq et al., Zahidah et al., and Thiyagarajan and Jamal 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 increases the 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., Joseph and Priya, Nath and Bhattacharjee, Tahera et al., Malaviya and Mishra, Mushhaq et al., Ali et al., and Anas et al.

The use of control (known antibiotics i.e., Sparfloxacin, Erythromycin, Fluconazole, Ampicillin, Tetracycline, Kanamycin, Streptomycin, Vancomycin, Chloramphenicol, Penicillin G, Cloxacillin etc) 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., Bansoda and Chavan, Nath and Bhattacharjee, Mushhaq et al., Balangcod et al., Zahidah et al., Anas et al., Taura et al., and Thiyagarajan and Jamal.

Study by Esmone et al. on methicillin-resistant S.aureus which used oxacillin as control for resistance and the strains were classified as resistant according to CLSI guidelines. The studies by Joseph and Priya and Tahera et al. made reference to controls with 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.
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<th>E</th>
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<th>M</th>
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M= Methanol, AC= Acetone, E= Ethanol, AQ= Aqueous

a=cold water, b=hot water, - = not evaluated

Table 1: Summary of results from selected article

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<th>AC ZONE OF INHIBITION (mm)</th>
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Figure 1: Total inhibition activity of all extraction solvent against test organisms

Figure 2: Activity index of extraction solvent for S. aureus
**Figure 3:** Activity index of extraction solvent for E. coli

**Figure 4:** Comparison of inhibition zone by different solvent

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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., 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]) as 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. coli* when 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. 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 Ojjan and Nihorimbere, Wongdoyo et al.

The result from the analysis of the antibacterial property of guava fruit by Tahera et al. on skin and core and that by Malaviya and Mishra 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 Omorie et al., Bansoda and Chavan, Balangcodd et al., Romasi et al., Taura et al., and Thiyyagarajan and Jamali revealed the presence of Carbohydrate, Tannins, Glycosides, Saponeins, 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., Iwu, Nadkarni and Nadkarni, Oliver-Beverley, Begum et al., Wyk et al., Ghosh et al., Chen et al. and Mathwally et al.

However, Romasi et al. recorded no trace of Triterpenoid when water was used as extraction solvent with no sensitivity from both organisms i.e. no zone of inhibition. Bansoda and Chavan also had no result for Terpenoid (methanol), Tannins and Saponeins under extraction by use of ethanol but recorded a low sensitivity from *E. coli*. Alkaloids were also reported missing in research by Thiyyagarajan and Jamali with ethanolic and Omorie et al. 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, Saponeins combined with Oleic acid, flavonoids, guajavarin, Quercetin, hexanal, 2-hexenal, 2,4-hexadienal, 3-hexenal, 2-hexenal, 3-hexenyl acetate and phenol, while β-caryophyllene, nerolidol.etc. (Nadkarni and Nadkarni, Paniandy et al., Joseph and Priya) however, no phytochemical analysis was carried out by Tahera et al. and Malaviya and Mishra. *S. aureus* showed higher sensitivity to extract from the fruit (skin and core) of Psidium spp 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. and this is in line with reports by Mitchel et al.; Ojjan and Nihorimbere which also found Proteins, starch, oils, glycoside, quercetin-3-O-β-D-(2′-Ogalloygluco-side)-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 sugars, saponins and oil with low concentration of steroids and terpenoids (Esimone et al.29). Similar composition was earlier reported by Begum et al.42 and Nadkarni and Nadkarni40. 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 oxygen25 has been indicated to contribute to choice of solvent. The increase in the use of methanol could be due to its polarity index (amphiphillic), 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.

REFERENCES


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