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RESEARCH ARTICLE

Extraction and Phytochemicals Determination of Traditional Medicinal Plants for Anti-microbial Susceptibility Test

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ABSTRACT

The study were conducted at Dilla University, College of Natural Sciences, Biological Sciences laboratories.

Background: Ethiopia is a country with many ethnic groups, cultures and beliefs which in turn have contributed to the high diversity of traditional health care knowledge and practices of traditional medicine from local growth plants, animals and minerals for various physical and mental disorders of human and livestock population that passed from generation to generation for centuries. Medicinal plants contributors to pharmaceutical, agricultural and food industries in the world. The use of medicinal plants in the industrialized societies has been traced to extraction and development of several drugs used in order to heal some diseases having inhibiting effect against pathogenic microorganism.

Objective: The main objective of this study was Extraction and Phytochemicals determination of traditional medicinal plants for anti microbial susceptibility test.

Methodology: The extraction and identification of some phytochemicals crude compound which used for antimicrobial susceptibility test from plant sample such as *Ocimum lamiifolium* (OL), *Croton maerosth* (Cm) and *Ruta chalepesis* (RC) were conducted. Plant samples are collected, powdered using mortar and pistil and extracted using ethanol and some susceptibility tests were performed to identify some phytochemicals compound.

Result: The main result of Antimicrobial activity test showed that the crude extract of OL has the highest zone of inhibition. The highest yield of crude extract (38.21%) was obtained from *Croton maerosth* (CM) which followed by *Ruta chalepesis* (RC) (32.43%). However, the lowest yield (28.37%) was obtained from *Oscpnum lamifolium* (OL).

Conclusion: Traditional Medicine is used by many people to managing numerous conditions; it's accessible and effective on antimicrobial activity. Therefore, it plays a significant role by reducing life-threatening ailments of people and other animals.

KEYWORDS: Extraction, Traditional Medicine, Phytochemicals determination.

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INTRODUCTION

Historically, learning via observation and experimentation indicates that plants have played as a major role for traditional medication and used to promote health of human beings. Traditional medicinal plants are used as remedies of safe for health, cost effective, almost free from serious side effect and also used as health practice, approaches, knowledge and beliefs. They are obtained from plant, animal and mineral based which used for spiritual therapies, being the plants are the most important source of medication. They used to apply in single or in combination to treat, diagnose and prevent illness and maintain well-being (WHO, 2001; Mazid, *et al.*, 2012).

The people did not have the scientific insight to explain the use of traditional medicinal plants are found in many of the healing cultures of the world based on the assumption that the appearance of plant may give clues to their medicinal properties and interpreted as God's signature on the plant. For example, red juice and sap, is associated with blood and menstrual elements and also yellow flower and alkaloid containing latex of some plants associated with bile and jaundice is crude extract used successfully to treat jaundice (Gurib-Fakim, 2006).

The traditional medicinal plant bioactive compounds are obtained mostly from plant leaves and used as an

alternative medication for the treatment of tonic diuretic, antiphlogistic and blood purifier and also used as remedy against chronic ulcers, chronic eczema, chronic nervous disease, chronic rheumatism, cholera amenorrhea, madness, piles and fistula. The powder of the dried leaves is often given with milk in mental disability for the improvement of the memory. The fresh juice of leaves is grown as alterative in jaundice, fevers and gonorrhoea. The same is also useful for children's in cutaneous diseases and for the improvement of nervous system (Mazid, *et al.*, 2012). Ailments have over the years been a scourge and a threat to mankind. People from different cultural backgrounds have used different herbal plants, plant extracts, animal products and mineral substances (Addae-Mensah, 1992) as the means to care, cure and treat ill-health, with disease prevention, and with health promotion (Curtis and Taket, 1996) since pre-historic times. Definition of traditional medicine by WHO as diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness (WHO, 2002; 2000).

There are a number of pathogenic microorganisms such as bacteria, fungi, viruses, etc are the main causative agents of infectious diseases that is a critical challenge to health and they are believed to be one of the main causes of increasing the rates of morbidity and mortality worldwide (Drusano, 2004). Various infections and disorders are caused by bacterial and fungal pathogens such as *Aspergillus*, *Bacillus*, *Candida*, *Cryptococcus*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Staphylococcus* and *Trichophyton* (Bibi *et al.*, 2011). The natural remedies and medicinal plants are the main resources for the physicians, especially in developing countries, depend on plants for medicines (Amabye and Shalkh, 2015). The importance of plants to homeopathy and modern medicine is correlated to their chemical constituents such as such as terpenoids, phenolics, alkaloids, flavonoids, amino acids, saponins, glycosides, diterpenes, triterpenes and their compatibility with the human body. It is expected that more than 30% of the worldwide sales of drugs is based mainly on plant products (Patwardhan *et al.*, 2004, De Fatima *et al.*, 2002). Plants of the family Rutaceae are a source of huge variety of natural products with antibacterial, antifungal, antioxidant, spasmolytic, antihelmintic, emmenagogue, antitumoral, analgesic, anti-inflammatory, and antidepressant activities (Raghav *et al.*, 2006, Di Stasi *et al.*, 2002). In many countries, medicinal plants are cultivated for its pharmacological and biological activity and it is widely used for treatment of gastric, diuretic, inflammation, headache and rheumatism disorders. Analysis of the chemical constituents of *R. chalepensis* extracts revealed that the aerial parts contain alkaloids, phenols, flavonoids, amino acids, saponins and furocoumarins (Kacem *et al.*, 2015). Therefore, conducting the present study on the title of extraction and phytochemicals determination of traditional medicinal plants such as *Ruta Chalepensis*, *Ocimum*

Lamiifolium and *Croton Maerosth* for Anti-microbial Susceptibility Test is important.

Globally : millions of people rely on traditional medicinal plants not only for primary health care, but also for income generation and livelihood improvement (WHO, 2002). Annual sales of herbal based medicines are ranged from 7.5-108 billion US\$ worldwide (Scherr, *et al.*, 2004). And in Canada annual market sales of medicinal plants reached 400 million US\$ in 2001 (WHO, 2005) and are growing at a pace of 15% annually (Lafreniere and Chenier, 1997). Traditional medicinal plants are widely used in different part of the world for curing diseases. They have maintained their popularity in developing world. These medicinal plants are also rapidly spreading in the industrialized countries. For instance, in china, about 30%-50% of the total medicinal consumptions was obtained from traditional herbal preparations in Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria are the use of herbal medicines at their home (Bannerman, *et al.*, 1993; WHO, 2003).

In Africa : up to 80% of the population uses traditional medicines for primary health care (WHO, 2003). Several African countries traditional birth attendants assist in majority of births using traditional plants (Bannerman, *et al.*, 1993; WHO, 2003). Traditional Medicine is assuming greater importance in the primary health care of individuals and communities in many developing countries (Peltzer and Mngqundaniso, 2008; WHO, 2002). These approaches to health care belong to the traditions of each culture, and have been handed down from generation to generation (WHO, 1996). Tribes, cultures and indigenous people of nations throughout the world have evolved system of traditional medicine for generations, and communities have found most of these medical practices valuable and affordable and still depend on them for their health care needs. The WHO estimates that about 60% of the world's people uses herbal medicine for treating their sicknesses and up to 80% of the population living in the African Region depends on traditional medicine for some aspects of primary health care (WHO, 2000).

In Ethiopia : Up to 80% of the population uses traditional medicine due to cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine and difficult access to modern health facilities. In 2000 only 9.45% of all deliveries in Ethiopia were attended by trained attendants and health workers. The rest were attended by traditional birth attendants or relatives (Lambert, 2001). Ethno botany is defined as the interaction of "local people with the natural environment: how they classify, manage and use plants available around them". In general, ethno botany is the scientific investigations of plants as used in indigenous culture for food, medicine, magic, rituals, building, household utensils and implements, musical instruments, firewood, pesticides, clothing, shelter and other purposes (Falsetto S., 2008)

Statement of the problem : Medicinal plants are played significant role in medication phytochemicals that extract from medicinal plants are used to cure different diseases

or ailments, but public does not use it properly because of lack of awareness on how to use traditional medicinal plant. Therefore, the main problems that initiate the researchers to undertake this research was that there is no study on public perception on the use of traditional medicine, extraction and phytochemicals analysis from medicinal plant for anti-microbial susceptibility test and still there are unknown plants and plant parts probability used as a medication to treat some diseases and also documented as remedies. In addition, there is no link between healers and modern health workers to exchange their knowledge on the method of preparation, route of administrations, effectiveness, safety as well as storage and awareness about the pharmaceutical use of traditional medicinal plants also to modify as modern drug usage.

Objective of study: the main Objective of this study was to extraction and phytochemicals determination of *Ruta Chalepesis*, *Ocimum Lamiifolium* and *Croton Maerosth* traditional medicinal plants for anti-microbial susceptibility test. Specifically, to identify the part of plant that is used for the preparation of traditional medicine, and to extract and identify some composition of chemical from crude extract of selected traditional medicinal plant. The main significance of the study is extraction and phytochemicals analysis from medicinal plant for anti-microbial susceptibility test, for documentation as remedies and also to create awareness and link between healers and modern health workers to exchange their knowledge. It may used as a basic information source about indigenous knowledge on medicinal plants preparation, part used, types of ailments, rout of administration, it may also used as a baseline for further studies in the area on medicinal plants and animals and to recommended conservation and protection measures of medicinal plants and animals biodiversity.

METHODS

Description of Study Area

Dilla town is located at Gedeo zone, southern nation and nationality people region of Ethiopia. The town has latitude and longitude 38⁰18'36"E and 6⁰24'30"N with an elevation of 1570 meter above from sea level and 360km distance from the capital city of Ethiopia, Addis Ababa. The climate of Gedeo zone is characterized as worm humid temperature. The Gedeo zone is endowed with two rain season from March to May and July to December with interruption of 3 to 4 dry season. The climate is suitable for abundant forest cover. The population of Dilla town was around 59,150, the total number of male 31,068 and the women is 28,082 (PS, 2012). Gedeo zone contain one hospital established by government. The main economic activity and source of income in the district is farming.

Antimicrobial Susceptibility Testing.

Antibacterial activity test : the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility testing (Bauer *et al.*, 1996). Media such as nutrient agar (NA) and nutrient broth (NB) was used as cultural medium. Bacterial inoculums were prepared by suspending the freshly grown bacteria in 5 ml sterile NB and the turbidity was adjusted to that of a 0.5 Mcfar land

standard. A concentration of 1-2x10⁸CFU/ml of bacterial inoculate was used. The media were poured onto 60mm diameter Petri plates (4mm agar thickness). A 0.1 ml of each bacterial suspension were obtained from nutrient broth (NB) and inoculated on to fresh media of nutrient agar (NA). The bacterial suspensions were uniformly distributed using swabs. Plates were allowed to stand for 15 min. Then, 6mm diameter disks were impregnated with the crude extract of medicinal plant. The impregnated disks were symmetrically placed onto the medium by using sterile tweezers. The plates were incubated aerobically at 28°C for 24hrs. Based on the method described by Ayoola *et al.* (2008) the plant leaf samples phytochemical compositions were analyzed.

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The following steps were used for extraction and phytochemical determination of selected traditional medicinal plant for antimicrobial susceptibility test

- ✓ Firstly the leaf part of medicinal plant were collected.
- ✓ Next to that dried the leaf of medicinal plant in the laboratory.
- ✓ Then grinding the leaf of each by mortal and pestil
- ✓ The powder measured by beam balance each 37g for three plant leaf extract.
- ✓ Then mixed with solvent ethanol by three separated flask and shakes by mechanical shaker for 24hr
- ✓ After that the solution was filtered by filter paper in each flask.
- ✓ Then evaporate the ethanol solvent separate from crude extract by rotator evaporation.
- ✓ Next to rotator evaporation the crude extract were kept in a tightly closed bottle in a refrigerator at 4°C until used for anti- microbial susceptibility testing.



Fig.1 : Powdered plant samples and ready for dissolving with in ethanol after measurement.

Sample A : *Ruta chalepesis* (Tenadam)

Sample B : *Ocimum lamifolium* (Damakese)

Sample C : *Croton maerosth* (Besana)



Fig. 2 : Powder Plants Samples Dissolved within Ethanol for Crude Extraction.



Fig.4 : Filtration process using filter paper.



Fig.3 : Process of Shaking a Mixture of Both Plant Samples and Ethanol in order to Shake Using Mechanical Shaker for 24 hr at Room Temperature.



Fig.5 : Process of separation of crud extract from solvent using rotary evaporator

RESULTS

Extraction and phytochemical contents determination of medicinal plants for Anti-microbial Susceptibility Test.

Different percentage yield of crude extract were obtained from *Oscpnum lamifolium* (OL), *Croton maerosth* (CM), and *Ruta chalepesis* (RC), selected medicinal plant. The percentage yield was calculated as following: percentage yield = (weight of crude extract /weight of sample) x100. The result of percentage yield for these some selected medicinal plants depicted in table 1. In this study, the highest yield of crude extract (38.21%) was obtained from *Croton maerosth* (CM) which followed by *Ruta chalepesis* (RC) (32.43%). However the lowest yield (28.37%) was obtained from *Oscpnum lamifolium* (OL). The result of this study, phytochemical content analysis of three medicinal plants such as *Ruta Chalepesis*, *Ocimum Lamifolium* and *Croton Maerosth* all indicated that the presence of glycosides, flavonoids, sterols, alkaloids,

protein, Resins, Lactones and tannins in different proportion.

The weight of all samples are 37g, but after rotary evaporator *Croton maerosth* higher weight of crude extract but *Oscpnum lamifolium* lower weight of crude extracts and the medium was *Ruta chalepesis*.

- In these study crude extract is high low inhibition zone.
- The crude extract very low is high inhibition zone of medicinal plants.

Some traditional medicinal plants contain some bioactive and phytochemical compounds. The present study has been shown that *Oscpnum lamifolium* (OL) *Croton maerosth* (Cm) and *Ruta chalepesis* (Rc) contain some phytochemical compound. The crude extract of all the studied plants leaves contain phytosterol, steroids and tannin phytochemical compounds which are bluish green, yellow with green fluorescence and yellow precipitate in color after screening have been conducted, respectively.

Table 1 : Percentage yield of crude extract for some selected Medicinal plants.

| List of tests | Some selected Medicinal plants | | |
|---------------------------|--------------------------------|------------------------|------------------------|
| | <i>Oscpnum lamifolium</i> | <i>Ruta chalepesis</i> | <i>Croton maerosth</i> |
| Mean of weight of samples | 37g | 37g | 37g |
| Weight of extracts | 10.5g | 12g | 14.14g |
| Percentage yield (%) | 28.37 | 32.43 | 38.21 |

Table 2 : The comparison of crud extract of medicinal plant and inhibition zone.

| Sample of medicinal plants | Crude extract | Inhibition zone |
|----------------------------|---------------|-----------------|
| <i>Oscpnum lamifolium</i> | Low | High |
| <i>Ruta chalepesis</i> | Medium | Medium |
| <i>Croton maerosth</i> | High | Low |

DISCUSSION

Extraction and Phytochemicals Determination of Traditional Medicinal Plants for Anti-microbial Susceptibility Test.

This study states that the presence of anti-microbial susceptibility test in *Oscpnum lamifolium* (OL), *Ruta chalepesis* (Rc) and *Croton maerosth* (Cm), the leaf extracted result indicated that the highest activity of *Oscpnum lamifolium* with inhibition zone. This show leaf of *Oscpnum lamifolium* has high ability to inhibit bacteria with appropriate solvent and phytochemical content composition indicated that the presence of glycosides, flavonoids, sterols, alkaloids, protein, Resins, Lactones and tannins. The study by Alhadi, *et al.*, (2015) stated that preliminary phytochemical screening of *Cordia Africana* showed that the presence of saponons, cumarins, tannins, triterpenes and flavonoids in the different plants parts and also noted that anthraquinones, glycosides and cyanogenic glycoside were absence from *C.Africana* plant parts. However, they reported a sterol only from the stem parts. Different phytochemical compounds were isolated from *Cordia sinensis* and these compounds includes flavonoids, saponins, sterols and sugars (Nawal, *et al.*, 2011). The study conducted by (vijayakumari, 2011) result shown that *Rotulaaquatic* contains alkaloids, flavonoids, phenols, saponins, terpenoids, Anthraquinones and anthocyanin.

The present result showed that the tested medicinal plants *Croton maerosth* has less inhabiting effect against bacteria than result reported by Alhadi, *et al.*, (2015) while they were conducted on leaves of *Cordia Africana*. Extract exhibited effects against most of the tested organisms with zones of inhibition ranging from (14-30 mm) and the largest inhibition against *Apergillus niger* give (30 mm). The stem of *C. Africana* extract exhibited effects against most of the tested organism with zones of inhibition ranging from (14-20 mm) and the largest inhibition against *Candia albicans* gives (20). The bark of *C. Africana* extract exhibited effects against most of the tasted organism with zone of inhibition ranging from (11-18mm) and the largest inhibition against *Bacillus subtilis* gives (18mm). The bark of *C.Africana* extract exhibited effects against most of the tested organism with zone of inhibition

ranging from (18-22mm) and the largest inhibition against *Sphylococcus aureus* gives (22mm) (Alhadi, *et al.*, 2015). The result of this study on phytochemicals contents determination of medicinal plants with the sample of *Ruta chalepensis* were almost similar with the result of Mohammed *et al.*, (2014), Lunga *et al.*, (2014), and Dahija *et al.*, (2014). Phytochemical content analysis of *Ruta Chalepesis*, indicated that the presence of carbohydrates and/or glycosides, flavonoids, sterols and/or triterpenes, alkaloids, protein and/or amino acids, Resins, Lactones and/or esters and tannins. On the other hand, saponin, anthraquinones, cardinolides, and oxidase enzyme were absent. The presence of variations in phytochemical groups in any plant can be used as promising support of possible presence of biological activities (Mohammed *et al.*, 2014, Lunga *et al.*, 2014, Dahija *et al.*, 2014).

CONCLUSION

The phytochemical compounds have been isolated from selected traditional medicinal plants such as *Croton maerosth* (Cm), *Oscpnum lamifolium* (Ol) and *Ruta chalepesis* (Rc). Generally, the highest crude extract was obtained from Cm which followed by Rc. However, the lowest yield was obtained from Ol. Among the extract obtained, the Ol showed the maximum zone of inhibition against gram positive bacteria isolated from water sample. Traditional Medicinal plants and animals are easily accessible in Ethiopia, cheaper, and effective in treating various diseases using plant products but safety conditions of the practice of the traditional medicine is not safe as compared with the use of modern medicine.

RECOMMENDATIONS

Based on the result of the study the following recommendations are forwarded:

- Plant resources are the main source of life on earth science. Thus governmental officials and NGOs should participate on conservation of medicinal plants
- The government, Non-Governmental Organizations (NGOs) and other stakeholders must see the need to invest in research, education, equipment and other infrastructure which will help make people accrue maximum benefit from traditional medicine.
- The traditional medicine are cheaper, easily accessible, effective to treated various diseases, using plant products but safety conditions of the practice of the traditional medicine is not safe as compared with the use of modern medicine. Because of most of traditional medicinal plants users are illiterate. So, it is recommended giving continuous training for healers concerning safety, efficiency dosage and other related issues of medicinal plants remedies to connect with modern medications.
- Studies are needed on the public perception on traditional medicinal plants and animals, types and parts used, method of preparation, route of administrations, safety as well as storage and the use of traditional medication on Ailments.

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AUTHORS' CONTRIBUTIONS

The participation of each author corresponds to the criteria of authorship and contributorship emphasized in the

[Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals of the International Committee of Medical Journal Editors.](#)

Indeed, all the authors have actively participated in the redaction, the revision of the manuscript, and provided approval for this final revised version.

COMPETING INTERESTS

The authors declare no competing interests with this case.

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