WOLKITE UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF BIOTECHNOLOGY

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL EFFECT OF CASTOR BEAN (*Ricinus commis*) EXTRACT AGAINST ENSET BACTERIAL WILT (*Xanthomonas campestris pathovar musacearum*)

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SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE BACHELOR OF SCIENCE (BS.C) DEGREE IN BIOTECHNOLOGY

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WOLKITE ETHIOPIA

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BY GROUP ONE MEMBERS

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LIST OF ABBREVAITIONS

Xcm pv. musacearum – Xanthomonas campestris pathovar musacearum

DMSO - Dimethyl sulfoxide

WKU - Wolkite University

Wt - weight

CSA - central statistics agency

SNNP - South Nation Nationality and Peoples

EBW - Enset bacterial wilt

R. communis - *Ricinus commis*

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ABSTRACT

Xanthomonas Bacterial wilt attacks enset at any stage, including full maturity. It is reported that up to 80% of enset farms are currently infected by Enset Xanthomonas Wilt. The available genetic diversity of the disease, natures, biology, epidemiology, virulence spectrum of the disease, the host-pathogen interaction, mode of transmission etc. has not been exhaustively studied. Therefore the objective of this study was to extract antibacterial compound from castor bean. Xanthomonas Campestries musacerum was taken from collected sample. The maximum amount of extract was 23gram. Phytochemical extraction and screening from castor bean plant and Xcm controlling efficiency of plant extract was done by dissolving the plant extract with hexane, methanol and chloroform at Wolkite University Molecular laboratory of biotechnology department. As a result inhibition zones are highly formed at extract that dissolve in distilled water while hexane, methanol and chloroform shows less inhibiton than distilled water and some phytochemicals(phenols, terprnoids, tannins, steroid, saponnins, and resin) were identified. Based on the present research finding castor bean extracted by distilled water was shown a maximum inhibition zone which score 1cm at WY1, WY3 and 35 Xcm strain. Due to this result we recommended that castor bean has an antibacterial activity and further advanced researches have to be done to the future to use castor bean as antibacterial agent to control the disease.

***Key words**: castor bean, phytochemicals, enset, Xcm*

1. Introduction

Enset (*Ensete ventricosum (Welw.) Chessman*) is a perennial, herbaceous, monocarpic and monocotyledonous crop in the family Musaceae (Westphal, 1975). Of the six up to nine commonly recognized species of *Enset, E. superbum* and *E. glaucum* grow wild in Asia while *E. perrieriin* in Madagascar and *E. gilletii, E.homblei* and *E. ventricosum* grow in eastern Africa (Simmonds, 1962*). E. ventricosum* is the sole cultivated member in the genus Ensete, and is cultivated exclusively in small holder farming systems in southern and south western Ethiopia (Simmons, 1956). Domesticated enset is cultivated at altitudes ranging from 1, 200 to 3,100 meters above sea level. However, it grows best at elevations between 2,000 and 2,750 meters above sea level (Quimio and Mesfin, 1996; Brandt *et al.,* 1997).

According to Admasu *et al.* (1997), three enset based farming systems have been identified based on the level of priority given to enset cultivation in different zones and regions in Ethiopia. Enset is the first important food source in Gurage, Kembata, Sidama, Gedio, Hadya, Jemjem and Arero zones. It is a second important crop as co-staple food in Wolaita, Gofa, Kafa zones and Yem special woreda. It is planted as the third most important food crop in Wollega, Illubabora and in some parts of Southern region.

According to CSA (2016), the total area covered by harvested enset in Ethiopia is estimate to be 56, 261.1 hectares, of them, the total area covered by harvested enset in Southern Nations Nationalities and Peoples Regional State (SNNPRS) and Oromyia region is 38,516 and 17,631.9 hectares, respectively. Enset is important in the Ethiopian economy, it is little investigated and remains an undervalued (i.e do not recognized how valuable or important) commodity crop. However, there are lots of biotic and a biotic problems threatening Enset production (Quimio *et al.,* 1996). Among the biotic constraints, diseases caused by bacteria, fungi, nematodes and viruses; mammalian pests such as porcupine, mole rat, wild pig and insect pests such as mealy bugs have been identified as serious problems. Of all the biotic constraints, bacterial wilt disease, which is caused by *Xanthomonas campestris pv. musacearum (Xcm*) is the most important disease affecting yield (Welde-Michael, 2000).

Xanthomonas campestris pathovar musacearum (Xcm) is the causal agent of *Xanthomonas* wilt. Enset bacterial wilt is known to cause severe damage, as it attacks and kills the plants at any growth stages, including full maturity (ready for harvest). Once the plants are attacked by the disease, especially at late maturity stage, it affects whole systems, and usually causing a maximum yield loss. A serious outbreak of the disease was reported by Westphal (Westphal E, 1975) with losses up to 70%. The results obtained from recent bacterial wilt disease assessment made in some Enset fields of the SNNPR, showed losses of up to 100% under severe damage (Shank R,Chernet, 1996). Many researchers (Anita S *et al.*, 1996; Tsegaye B *et al.*, 1998: Endale *et al.*, 2003) reported that both the area and productivity of Enset is declining continuously due to this disease.

1.1 Statement of the problem

Since 1968 researchers from Ethiopia and abroad conduct different research on *Xanthomonas Campestries musacerum (Xcm)* like; Biochemical based characterization of *Xanthomonas Campestries pv. musacerum*, sanitary control methods to eradicate the disease and Xcm resistance enset clone screening. Moreover different researchers recommend *Xcm* resistant cultivars, however those cultivars recommend as resistance not be resistance when testing it by other areas *Xanthomonas Campestries musacerum* suspected isolate. Moreover, *Xanthomonas Campestries musacerum* cause enset systemic disease due to this reason the disease controlling system is very difficult until yet. Hence, using phytochemicals for intracellular controlling of the systemic pathogens is recommended option.

1.2 Significance of the study

The bacterial genus *xanthamonas* consists of several species of economic importance, among which *xanthamonas campestris pv. muscarium (xcm),* the cause of enset wilt is the most important in tropical Africa. Enset is the most important edible plant in most area of Ethiopia but it is affected by this bacteria *(xcm*). The significance of this study is to extract antimicrobial compound from castor bean to against enset infection by *Xcm.*

1.3 OBJECTIVES:

1.4 GENERAL OBJECTIVE

- To screen phytochemicals and to determine the antibacterial effect of castor bean extract against to Enset bacterial wilt caused by *Xanthomonas campestris pv.Musacearum(Xcm)*

1.5 SPECIFIC OBJECTVES

- To extract anti *Xanthomonas campestris pathovar musacearum* compounds from castor bean plants species by using different solvent
- To screen the phytochemical compounds which found in castor bean
- To know antibacterial(*Xcm)* potential of the castor bean extract

2. LITERATURE REVIEW

2.1 Crop Description

2.1.1. Origin, taxonomy and distribution of enset

Ethiopia is enset's center of origin and holds a large number of enset geremeplasm collections from several geographical regions (Vavilove, 1951; Mikias, 2014). There have been efforts to understand local production practices and improve the conservation and use of the genetic resources of enset in order to enhance the mostly under exploited potential of this crop. Enset (*Ensete ventricosum*) consists of 6-7 species (Simmonds, 1962; Pursglove, 1972). Taye (1984) has also stated that 7 up to 8 species are known in the genus that means; stile within ambiguity about number of species in the genus Ensete.

In the past *Enseteventricosum* was cultivated only in the south and south-western parts of Ethiopia, but the recurrent droughts have led to the expansion of enset cultivation to other parts of the country (Brandt *et al*., 1997). A wide adaptation within the species to altitude, soil and climate has allowed widespread cultivation in western Bale, south-western Oromia including south and east Shewa, Jima, Illubabor and Welega (Shank, 1994).

2.1.2 Morphological description

Enset is evergreen plant and it is composed of corm, pseudo stem and leaf as well as bended inflorescence during its maturity. A mature enset plant has an average height of 4–8 meters, but it could reach as high as 10 meters depending on the cultivars used and site conditions. The average basal diameter of its dilated pseudo stem is 0.5-1.0 meter. The pseudo stem, which is composed of leaf sheath, has a length of 1.0 to 2.5 meters in fully matured plants. Leaves are large with oblong blades and often long free petiole. The inflorescence grows from the center of plant, and fruits are small with large black non edible seeds (Bizuayehu, 2002). The vegetative growth habit of enset is similar to banana (Musa spp.) plants, but enset is not grown for the fruits; these contain mostly large and very hard seeds (Karlsson *et al*., 2013).

2.1.3 Ecology, agronomy and production status in Ethiopia

Enset performs well in areas where the natural environment is steppe (Negash *et al.,* 2012) and naturally occurs in forests and in humid areas (Baker and Simmonds,1953) it is suitable to grow

in a wide range of climates, thus in large geographical areas. Temperature plays a significant role in the growth rate of enset. Accordingly, at the altitudinal ranges of 1500-2300 meters (WoynaDega areas) where mean annual temperature is 15-20°C, enset grows fast and reaches full maturity in 5 to 7 years. On the other hand, in the high altitudes of 2300-3100 meters (Dega areas), where mean temperature drops to 10-15°C, it takes 8-10 years and sometimes up to 16 years (Shank and Ertiro, 1996) to reach full maturity. Enset need different agronomic practices like hoeing, weeding, and repetitive transplanting. Enset thrives best in fertile, well drained soils of moderately acidic to alkaline nature (Admasu Tsegaye, 2002).

Enset cover about 65% of the total crop production in the southern region of Ethiopia and productivity is very high compared to other crops but varies depending on edaphic factors, altitude, cultural practices and varietal differences (Genet *et al.,* 2004). According to CSA (2014) report the production status of enset in Oromia region the estimated number of Enset trees harvested, in the 2013/2014 agricultural year, estimated were 34,461,351.0. Thus, the total produce in the form of Amicho, Qocho, and Bula was 7,061, 725.87; 8,303,792.24 and 320,991.67 quintals respectively. For instance at Jima, Borena,

West Arisi and south west shewa the estimated number of enset tree harvested was 3,440,966.00; 1,495,355.00; 17,294,385.00 and 1,929,028.00 respectively. At SNNP Region the estimated number of enset tree harvested was 96,023,384.00. From thus total harvested enset tree produce Amicho, Qocho and Bula were 24,079,056.78; 26,410,479.01 and 904,896.31 quintals respectively. From the region at Gurage, Hadiya, KmbataTembaro and Sedama the estimated number of enset tree harvested was 3,760,845.00; 2,830,620.00; 1,211,004.00 and 56,320,910.00 respectively.

2.2 Economic and Ecological Importance of Enset

Enset is an important multi-purpose and drought-tolerant crop, used for food and In terms of edible dry weight and energy, enset gives higher yield than other crops cultivated in Ethiopia (Admasu and Struik, 2001), fiber (Tsehaye and Kebebew, 2006) and traditional medicine (Nyunja *et al.,* 2009). The food is rich in starch, is a good source of calcium and iron (Atlabachew and Chandravanchi, 2008) and has overall nutritive values similar to potato (Mohammed *et al.,* 2013). A mixture of scraped leaf sheath and pulverized corms, after

fermentation in a pit, results in production of Qocho. Qocho is the main product consumed after making a pancake-like food. Bulla is another important food product from enset produced from solidified liquid after dehydrating a fresh mixture of scraped leaf sheath and pulverized corms. Bulla is consumed mainly as porridge, in gruel and as crumbled forms. Corms of some clones are cooked and consumed similar to roots and tubers of other crops. Processed products such as Qocho and bulla are sold in small town markets and also transported to the cities (spring, 1996). Leaves, as a wrapping material, and fibre are additional sources of income. Fibre, a by-product of enset in food processing, is a valuable raw material for household usage. Local fibre factories use this as an import substitute because the quality of ensetfibre is equal to that of abaca and better than sisal (Bezuneh, 1996). Enset fiber accounts for more than 30% of the Ethiopian fibber production and its strength is equivalent to the fiber of Abaca (Brandt *et al.,* 1997).

Enset plantations prevent soil erosion and conserve soils, hence, contributing to the sustainability of the farming system. Enset contributes positively to the local environment by improving the nutrient balance and increasing the fraction of organic matter in soil (Asnakech, 1997). Enset is suitable for agroforestry and it is part of farming systems with high biodiversity which is environmentally sustainable (Bizuayhu, 2008; Negash *et al*., 2012), which is suggested for land improvement.

2.3 Problem Associated with Enset Production

The sustainability of Enset agriculture is threatened by fungal, bacterial and viral diseases. Bacterial wilt of Enset which caused by *Xanthomonas Spp.* is the most important disease affecting Enset yield (Yirgou and Bradbury, 1968; Gizachew *et al.,* 2008; Ashagari, 1985; Quimio and Tessera, 1996).There are also other production constraints such as, lack of *xanthomonas* disease resistance enset cultivars and due to variable nature of the disease there is no effective disease control mechanism. The disease also have varied nomenclatures and genetics constitute, it is also proposed as *Xanthomonas vasicola pv. Musacearum* (Aritua *et al.,* 2008).

Accurate and reliable controlling mechanisms are necessary and important for developing management measures. In development of control strategies against *xanthomonas* diseases, the

main problem is the presence of different strains within a given pathogen population, systemic disease causing system and absence of intracellular disease control technology.

Figure1: Cream to pale yellow bacterial ooze appears soon after the pseudostem is cut

2.4 Castor bean

Castor bean (*Ricinus communis*) is an indeterminet, non-edible oil seed crop grown in low rainfall regions of semi-arid tropics and sub-tropics. *Ricinus communis L. (R.communis*) belongs to family *Euphorbiceae*, is a soft wooden small tree, widely spread all through the tropics and temperate regions of the world (Sayono. S, *et al* 2020).present research work reveals the in vitro antimicrobial properties of methanol, hexane, chloroform and distilled water extracts of the leaves of *R. communis L.* against *xanthomonas*.

2.4.1 Morphology of Castor bean

The castor bean (*Ricinus communis*) is a tropical perenniall shrub and field crop originated in Africa, but has now been introduced worldwide and is widely cultivated (Chanetal.,2010). It can be cross and self-pollinated, and studies have revealed low levels of genetic variation among castor bean germ plasm worldwide (Allan *et al*., 2008; Fosteretal., 2010). Because of then early uniform ricinoleic acid content of castor oil and its unique fatty acid properties, castor beans are a valuable oil seed crop for the cosmetics industry, specialty lubricants ,and biomedical and specialty chemical applications. *R. communis* has also been proposed as a potentials of biodiesel feedstock because of its high seed oil content (DaSilva *et al*., 2006), and the case with which it can be cultivated in unfavorable crop-growing environments has contributed to its appeal as a

crop in tropical developing nations. Furthermore, the castor plant is commonly cultivated in many counties for its leaves to feed the Erisilk worm (Attacus Cynthiaricini Boisduval), which provides Erisilk, a high-quality natural protein fiber. *R.communis* is produced in about 30 countries for commercial purposes, among which India, China and Brazil account for >90% of the world's production (Severino *et al.*, 2012). Mechanized castor production is possible and needs to become mandatory to sustain or increase global castor production. Because the three main countries producing castor are experiencing rapid economic and social development, the labor required for traditional castor production has become expensive (Severino *et al*., 2012). Currently, only limited areas of castor production are fully mechanized because of the lack of dwarf-internode and commercial cultivars (Baldanzi *et al.,* 2003). Therefore, the main challenge in developing new cultivars is the adaptation of castor plants to mechanical harvesting. The development of appropriate new castor cultivars should be enhanced by improved knowledge of the genetics and molecular biology of the species. Mutation breeding has shown that irradiation of castor seeds and seedlings produces mutants with desirable characteristics including semi-dwarfs with higher yield potential and earlier maturity (Sujatha *et al.*, 2008)

Figure 2: morphology of castor bean;

2.4.2 Antibacterial activity of caster bean

Plants contain a wide variety of active compounds that exhibit antimicrobial and larvicidal activities. In addition, due to the fairly recent surge in the antibiotic resistance of pathogenic microorganisms and increasing consumer concerns regarding the negative health impact of synthetic preservative, there has been a dramatic increase in the application of natural antimicrobials of plant origin (T Islam *et al*., 2010) Besides the antibacterial, antifungal and anti-inflammatory activities, many essential oils and plant extracts have shown to possess antioxidant, anticancer, anti conceptive, anti phlogistic and antiviral activities).

The efficiency of the essential oils and plants extracts depends on its chemical composition which itself depends on the genotype of the plants as well as on the environmental and agronomic conditions. The presence of natural products such as terpenes, alkaloids, flavonoids, coumarins and other secondary metabolites support the popular uses of these plants in medicines and integrated pest management projects. Essential oils are composed of mixtures of volatile secondary metabolites commonly concentrated in different plant organs(A.A *et al*., 2013). Many tropical and subtropical plants have shown to possess larvicidal and antimicrobial activities. Several research works have also established the larvicidal potential of plant extracts and essential oils obtained from different parts of a variety of plants against larvae of Diptera. The insecticidal effects of plant chemicals vary not only according to plant species, mosquito species and plant parts, but also to extraction methods used in experiment(Z.Zarai *et al.,* 1012).

The castor bean plant, *Ricinus communis L. (Malpighiales: Euphorbiaceae) (R. communis*), is grown extensively throughout tropical regions of the world with wide applications in industry and agriculture. It is an important oilseed crop that produces oil rich in ricinoleic acid. The various solvent extractions prepared from the different parts of the plant have been reported to possess medicinal properties viz., hepatoprotective, anti-diabetic, laxative, anti-fertility, antimicrobial, analgesic, antihistaminic and anti-inflammatory(R.S *et al.,* 2012). Castor plants grow widely in Mauritius and this work was undertaken to evaluate the plant's dual bio-efficacy effects, namely, antimicrobial and larvicidal activities.

2.5 Secondary metabolites

Plants produce a large and diverse array of organic compounds that appear to have no direct functions in growth and development i.e. they have no generally recognized roles in the process of photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation (Hartmann, 1991). They have a very restricted distribution than primary metabolites in the whole plant kingdom i.e. they are often found only in one plant species or a taxonomically related group of species. Also, they are a highly active group of molecules with a wide range of anti-microbial activity against both fungi and bacteria (Brooker *et al.,* 2008). There are three major groups of secondary metabolites viz terpene, phenolics and N and S containing compounds. Terpenes composed of 5-C isopentanoid units, are toxins and feeding deterrents to many herbivores. Phenolics synthesized primarily from products of the shikimic acid pathway, have several important defensive role in the plants. Members of the third major group i.e. N and S containing compounds are synthesized principally from common amino acids (Van Etten *et al.,* 2001).

2.5.1 Terpenes

Terpenes constitute the largest class of secondary metabolites and are united by their common biosynthetic origin from acetyl-coA or glycolytic intermediates (Gerhenzon *et al*., 1991; Grayson, 1998; Fraga, 1988; Croteas, 1988; Loomis and Croteas, 1980; Robinson, 1980). A vast majority of the different terpenes structures produced by plants as secondary metabolites that are presumed to be involved in defense as toxins and feeding reddish brown colour at the interphase indicates the presence of terpenes and steroids (Harbone, 1973). deterrents to a large number of plant feeding insects and mammals (Gershenzon and Croteau, 1991).

2.5.2 Phenolic compounds

Plants produce a large variety of secondary products that contain a phenol group, a hydroxyl functional group on an aromatic ring called Phenol, a chemically heterogeneous group also. They could be an important part of the plants defense system against pests and diseases including root parasitic nematodes (Wuyts *et al*., 2006). Elevated ozone (mean 32.4ppb) increased the total phenolic content of leaves and had minor effects on the concentration of individual compounds

(Saviranta *et al*., 2010). A deep blush green solution indicates the presence of phenols (Odebiyi and Sofowora, 1990).

2.5.3 Tannins

Tanins: It included under the second category of plant phenolic polymers with defensive properties. Most tannins have molecular masses between 600 and 3000. Tannins are general toxins that significantly reduce the growth and survivorship of many herbivores and also act as feeding repellents to a great diversity of animals. In mammalian herbivores, they cause a sharp, astringent sensation in the mouth as a result of their binding of salivary proteins. Mammals such as cattle, deer and apes, characteristically avoid plant with high tannin contents (Oates *et al*., 1980). The defensive properties of tannins are generally attributed to their ability to bind proteins. Protocatechllic and chlorogenic acids probably have a special function in disease resistance of certain plants. They prevent smudge in onions, a disease caused by the fungus *Colletotrichum circinans* and prevent spore germination and growth of other fungi as well (Vickery, 1981; Butt and Lamb, 1981; Mayer, 1987). It is thought by some that chlorogenic acid and certain other related compounds can be readily formed and oxidised into potent fungi staticquinones by certain disease resistant cultivars but less readily so by susceptible ones. brown precipitate which indicates the presence of tannins (Sofowora, 1993).

2.5.4 Flavinoids

Flavonoids: One of the largest classes of plant phenolic, perform very different functions in plant system including pigmentation and defense (Kondo *et al*., 1992). Two other major groups of flavonoids found in flowers are flavones and flavonols function to protect cells from UV-B radiation because they accumulate in epidermal layers of leaves and stems and absorb light strongly in the UV-B region while letting visible (PAR) wavelengths throughout uninterrupted (Lake *et al*., 2009). In addition, exposure of plants to increased UV-B light has been demonstrated to increase the synthesis of flavones and flavonols suggesting that flavonoids may offer a measure of protectionby screening out harmful UV-B radiation (Caldwell *et al*., 1983; Saviranta *et al*., 2010).Either cream or light yellow colourations confirms the presence of flavonoids (Harbone, 1985).

3 .MATERIAL AND METHOD

3.1 Study area

This study was conducted at Wolkite University which is located 170 KM away from South West direction of Addis Ababa at altitude and longitude 8°17′N 37°47′E/8.283N 37.7835E With elevation between 1910 and 1935m above sea level. The mean annual temperature is 14 to 24°C.

3.2 Sample Collection AND PREPARATION

Castor bean plants as a source of phytochemicals were collected from WKU compound. The collected sample was washed with distilled water and shade dried completely. The dried plant sample was grinded using a grinder.

Figure 3: castor bean leaf and powder preparation

***Xanthomonas campestris pv. Musacearum* Sample preparation**

Xcm sample pathogen sample was taken from already isolated sample, while based on recorded *Xcm* sample collection passport information *Xcm* sample was collected from different *Xcm* hot spot woredas of gurage Zone. The pathogen *Xanthomonas campestris pv*. *musacearum*, was refreshed by selective media i.e. Yeast Dextrose Calcium Carbonate (YDC) agar media (yeast 10 g, dextrose 20 g, calcium carbonate 20 g and agar 15-20 g per 1000 ml of sterile distilled water, when targeted to prepared one litre). Calcium carbonate was added after cooling of the other ingredients. It was streaked on solid surface of YDC plates and incubated at 28°C for three days as done by Blanchard and Talter (Westphal E, 1975). After 72 hours of incubation, colonies were shown light yellow mucoid growth, typical of Xcm was transferred to Nutrient broth and maintained at 4°C in refrigerator for further studies (Mesfin *et al.*, 2008).

3.3 Phytochemical extraction Procedure

After collection of botanical plant parts extraction of active compound was proceed. Extraction process was performed by using shaker extraction with four different solvent. 45g powder plant sample was dissolved using 450ml solvents of Methanol, hexane, Chloroform and Distilled water. The extraction was made by shaker methods with in 72hrs. The solvents were removed using Water bath.

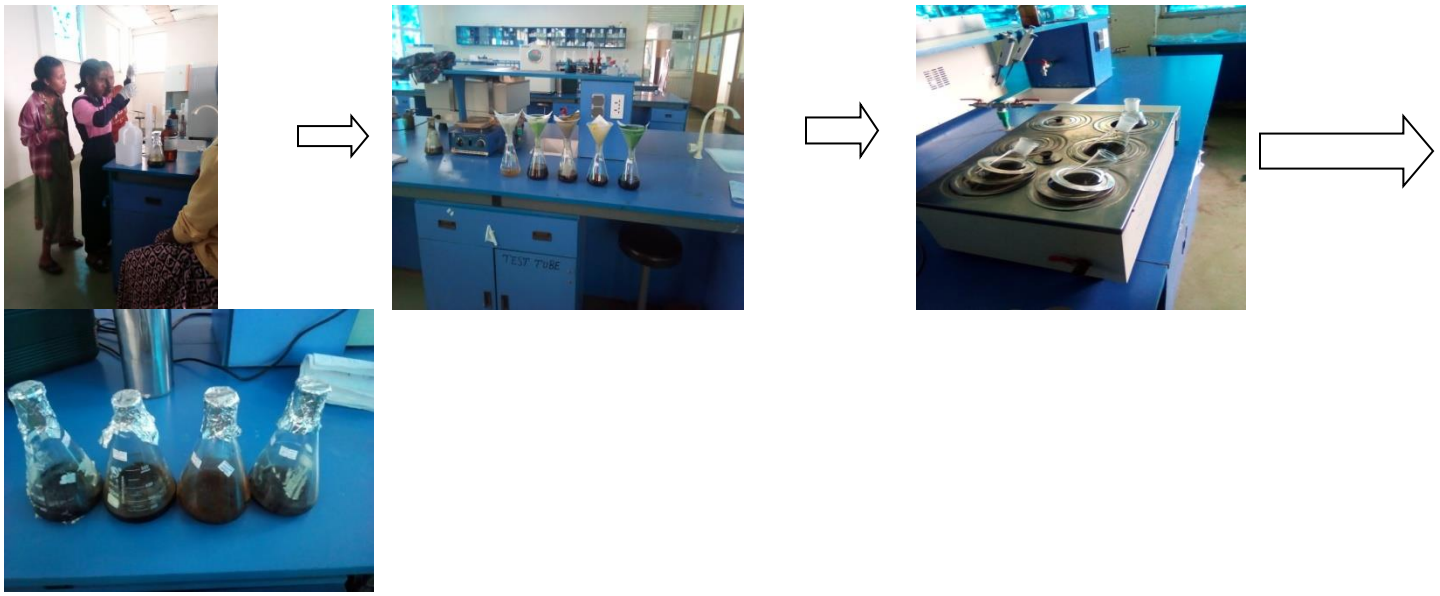

Figure 4: Phytochemicals extraction procedure via using Castor bean leaf sample

3.4 Phytochemical Screening

The extracts obtained were subjected to phytochemical test screening using standard methods. The phytochemical screening for major constitute was undertaken using different phytochemical screening test for the presence resins, flavonoids, phenols, saponins, terpens, tanins and steroids.

Test for Resins

Two milliliters of acetic anhydride was added to 2.0ml of the extract and a drop of concentrated sulphuric acid was also added. The observation of purple colour,readly changing to violet indicates the presence of resins .

Test for Flavonoids

A few drops of 10% of lead acetate solutionwasaddedto 2.0ml of the extract in a test tube. The observation of either cream or Light yellow colourations confirms the presence of flavonoids .

Test for Phenols

Two milliliters of extract was added to 2.0ml of ferric chloride. A deep blush green solution indicates the presence of phenols.

Test for Saponins

Five milliliters of distilled water was added to 2.0ml of the extract in a test tube. This was shaken vigorously after which a few drops of olive oil was added. Formation of an emulsion will indicate the presence of saponins. Also the formation of persistent foams during the concentration of plant extract is reliable evidence that saponins are present .

Test for steroid and Terpenesone

One milliliters of acetic anhydride was added to 2.0ml of the extract and then concentrated sulphuric acid was carefully added down the side of the test tube. An observation for reddish brown colour at the interphase indicates the presence of terpenes and steroids.

Test for Tannins

A few drops of lead acetate solution were added to 2.0ml of the extract. The resulting solution was observed for Brown precipitate which indicates the presence of tannins.

3.5 Antibacterial Activity Assay

The antibacterial activity of the various extracts of botanical plant produced by different solvents were determined by the agar walls diffusion method. The antibacterial activity assay was conducted on Mueller Hinton agar cultured *Xanthomonas campestris pv. Musacearum.* Antibiotic CIPRO disc used as positive control and DEMSO universal solvent used as negative control.

3.6 Extract Percentage yields

The percentage of the extracts were determined as percentage of the weight of the extracts to the original weight of the dried sample used, using the formula(Sheneni victory Duniya, *et al.* 2018).

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} * 100$$

4. RESULT AND DISCUSSION

4.1 Antibacterial activities of castor bean extract that dissolve in different solvent

In order to determine the antibacterial efficiency of castor bean extract (leaf sample) with different solvents the growth inhibition zone of ten *Xcm* isolate were measured. The results indicated that as compared to negative control (DEMSO) the extract by different solvent showed significance inhibition performance on all targeted *Xcm* isolate While the inhibition potential of castor bean extract via all tested solvents was less efficient as compared to positive control(CIPRO). As shown in Table 1 & Figure 5.

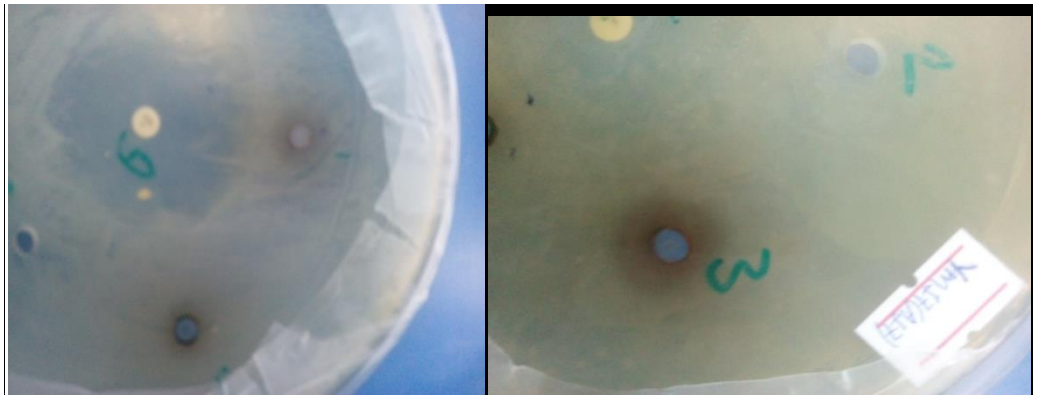

Figure 5: Castor Bean Extract against *Xcm* and created Inhibition zone

Table 1 the measured(in cm) diameter of inhibitory zone

Strains of Xcm	Hexane	DMSO	Distilled Water	Methanol	Chloroform	Control
TZ-11	0.7	-	0.8	0.4	-	3
WY1	-	-	1	-	-	3
28	-	-	0.7	-	-	2.3
29	-	-	0.5	-	-	3
YN16	-	-	0.5	0.3	-	2.5
YM17	-	-	0.9	-	-	2.3
WY3	-	-	1	-	0.5	2.5
XX2	0.2	-	0.4	-	0.3	2
XYS	0.6	-	0.5	0.5	-	2.5
35	-	-	1	0.7	-	2.5

Alternative medicine is gaining much attention to fight the problem of *Xcm*. A variety of plants or plant products are being paid renewed interest to explore for their anti-microbial activities in a more scientific and systematic manner. The present study was investigated the antibacterial potential of castor bean extract against ten strains of enset pathogenic bacteria. The result clearly indicated that, the antibacterial activity of castor bean extract was against gram negative bacteria strains used in this study. The current finding was indicated that, the test sample of castor bean had antibacterial activities. As shown in Table 1 & Figure 5 Maximum inhibition zone (1cm diameter) was recorded on Castor bean extracted with distilled water on *Xcm i*solate``WY1, WY3 and isolate 35`` However other *Xcm* isolate were had a significance variation for hexane, methanol, distilled water and chloroform dissolved extract; the variation was between solvent to solvent. The inhibition zone recorded were (1, 0.9, o.8, 0.7, 0.6cm) on the strain (WY3, WY1, TZ-11, YM17, 28) dissolved in solvents methanol, distilled water, hexane. Among solvents distilled water was had effectively against all pathogens. Most of tested extract of castor bean by different solvent revealed that, good *Xcm* inhibiting ability except on few isolate.

Distilled water extracts of the castor bean was most effective against all strains of *xanthomonas campestris pathovar musacerum.*

4.2 Identification of phytochemical constitute

The Phytochemical analysis of methanol, hexane, distilled water the extract of castor bean leaves indicates existence of resin, tannin, steroid, phenol, saponin, flavonoid and castor terpenoids. While for chloroform due to experimental error it was not done. Similarly others reported the presence of those phytochemicals on Daturametelplant dissolved by those solvents (Dixon Dhawan and Jeena Gupta; 2017)

Table 2.types of phytochemicals that are found in the plant castor bean extract dissolved with different solvents

Solvents	Resin	Tannin	Steroid and terpenoid	Phenol	saponins	flavonoid
Hexane	✓	✓	✓	✓	✓	✓
Methanol		✓	✓			
Distilled water	✓	✓	✓	✓		

The observed activity of castor bean could be due to its phenols, flavonoids, tannins, saponins, resin, steroid and terpenoids. Contents which have been reported to display anti bacterial activities for various microorganisms (kreicbergs *et al.*, 2015; Almedia *et al*., 2006).

Figure A figure B figure C

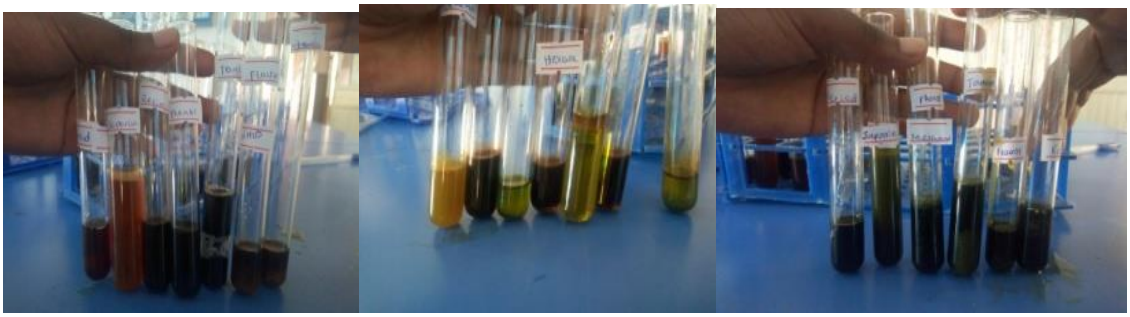

Figure A result for hexane figure B result for distilled water figure C result for methanol

4.3 **Effects of Different Solvents on Extraction Yield**

Water and organic solvents (methanol, chloroform, and hexane) were studied for their effects on the extraction yield of *caster bean leaf sample*. Results showed a significant difference in the extraction yield using different solvents. Among solvents tested, Methanol resulted in the highest extraction yield (51.1%), followed by Hexane (28.89%), distilled water (15.56%), and chloroform (10%)(Table 3) Indicating that the extraction efficiency favors the highly polar solvents than water.

Table3. Effect of different solvents on extraction yield

solvents	Weight of plant(in gram)	Weight of extract(in gram)	% yield
hexane	45	13	28.89
methanol	45	23	51.1
chloroform	45	4.5	10
Distilled water	45	7	15.56

5. CONCLUSION AND RECOMMENDATION

Large numbers of medicinal plants have been extracted, fractionated, and compounds isolated successfully. In addition, compounds obtained were tested for biological or pharmacological activity, and in most cases, they were found to be active. Nonetheless, the rate of success and the authenticity of these findings depends on the accuracy in selection of solvents, selection and proper execution of extraction methods, phytochemical screening, fractionation, and identification techniques. Lastly, proper understanding and implementation of these techniques are indispensable. Advancement and modification of these methods periodically will ease research processes and improve the outcome. The present study confirmed the antibacterial potential of castor bean extracts. The antibacterial activities of these natural products against the *Xcm* indicate their potential as sources of antibacterial agent that can be used against *Xcm*. Thus, According to the study we recommend that castor bean extract has an antibacterial activity. Such developments in the use of natural products from plant in disease control will minimize or avoid environmental and health hazards caused by synthetic chemical pesticides.

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