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PRELIMINARY STUDY ON THREE ERITREAN PLANTS WITH ANTIBACTERIAL ACTIVITY

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ABSTRACT

The aim of this study is to evaluate the antibacterial activity of *B.papyrifera, S.singuena*and *T.emetica* on selected microorganisms, *E.coli* (gram negative) and *S.aureus* (gram positive) with crude extracts at different concentration. The crude extract of each plant was prepared by cold extraction using three solvents of different polarity (distilled water, methanol (95%) and petroleum ether). The anti-bacterial sensitivity test was carried out by well method at different concentration of (25, 50, and 100) mg/ml. The results have shown that all the plants have significant anti-bacterial activity with methanol and water extract of the plants, but no any activity was shown by petroleum ether extracts of all the plants under study. Therefore the methanol and water extract of the selected plants have significant activity against *E.coli* and *S.aureus*.

Key words: *Boswelliapapyrifera, Sennasinguenna, Trichelliaemetica,* Antibacterial, Methanol extract, Water extract and petroleum ether extract

INTRODUCTION

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicines. The use of plant based medicine is a popular health care approaches in most parts of the world and is the most common form of treatment in many developing countries. Even in this time of domination of manmade materials, plants and plant products are still in greater demand. The use of plants and their extract for healing by herbalists, traditional healers and other specialists was the main method of treating various illnesses before the advent of modern medicine. Also in this period of time this continues specially in the rural areas. This is common in Eritrea specially in case of the healing of fractures, wounds and other pains. The skills of healing with herbs is acquired informally and improved up on normally. The present manual of natural medicines seeks rather to justify their uses on a sound basis of accurate chemical analysis and precise elementary research. Until now medicinal herbs have come down to us from time as processing only traditional value and as exercising merely empirical effect. Their selection has been commended solely by shrewd discrimination and by practice over successive centuries. But today a closer analysis in the laboratory and advanced skill provided by experts have resolved several plants into their component parts and have chemically determined the medicinal nature of these parts both singly and collectively. So that the study and the practice of curative medicinal herbs may now fairly take rank as an exact science and may command the full confidence of the sick for supplying trustworthy aid and succour in their times of bodily need.

LITERATURE REVIEW

For these selected plants *Boswelliapapyrifera, Sennasinguena and Trichelliaemetica* there is no any scientific report for their antibacterial activity.

1. Boswelliapapyrifera

Botanical description: *Boswelliapapyrifera* belongs to a tropical family called *Bruceraceae.* This is distinguished by the presence of resin ducts in the bark. The bark is whitish to pale brown, peeling off in large flakes; slash red-brown and exuding a fragrant resin. *Boswelliapapyrifera* is a *monocious* species with sweet scented flowers which are white to pink, arranged on long red flower stalks, in loose panicles at the end of branches.

Distribution and Habitat: *Boswelliapapyrifera is* one of those species with multiple economic in many parts of Africa. It is found in Eritrea, Ethiopia, Nigeria, Cameroon, Central African Republic, Chad, Sudan, and Uganda. In Eritrea it is dominant on steep rocky slopes of western escarpment, like Tsebab, Badime, Halibmentel, Mereb River, Shilalo, Augaro, Jengeren etc.

Uses:

Frankincense production: Frankincense constitutes 3-8% volatile oil, 60-70% alcohol-soluble resin and 27-35% water soluble gum.

Traditional medicine: Various plant parts and products are utilized for traditional medicinal purposes. The leaves and roots of the species are used against lymphadenopathy while the resin is used as a febrifuge.

2. Sennasinguena

Botanical description: *Sennasingueana* is a shrub or a tree 1-15 m high; branch glabrous to densely pubescent crown open; bark reddish, becoming grey-brown and rough with age.

Distribution and Habitat: *S.singueana* is a species of the drier tropical Africa regions. In Africa it distributed along Native: Angola, Botswana, Comoros, Eritrea, Ethiopia, Kenya, Malawi, Mozambique, Namibia, Tanzania, Uganda, Zambia, and Zimbabwe. In Ethiopia and Eritrea, it is a component of the mid- and highland dry evergreen forests. In Eritrea it grows around river Anseba, Mensae, Halhal, Karneshim, Dekemhare, Segenaiti, Dimbezan, Halibmentel etc.

Uses: *S.singueana* has many medicinal uses throughout Africa. Traditionally used for the treatment of a form of skin cancer and the root bark is used against convulsions, gonorrhoea, bilharzia, heartburn, stomach-ache, constipation, wounds and snake bites. The ash from the burnt roots mixed with porridge provides a remedy for stomach pains. Scientific reports indicate that the plant has anthelminthic properties, antiprotozoal activity against cestodes of *Hymenolepisdiminuta*, antiplasmodial, antinociceptive, antipyretic, antioxidant, Hepatoprotective properties, antiulcer effects and reduce both gastric free-HCl and total acids.

3. Trichiliaemetica

Botanical Description *Trichiliaemetica* grows along streams and rivers as well as in woodland, coastal forest, mountain forest and savannah. The growth rate is fast, maximum height is 8-20m. The dark grey to brown bark is smooth but can become rougher as the tree ages.

Distribution and Habitat:*Trichiliaemetica* is found in sub-Saharan Africa from Senegal to the Red sea, *Trichiliaemetica* prefers a sunny or semi-shaded position, *Trichiliaemetica* grows extremely fast, it can however withstand long periods of drought when mature. In Eritrea it is mainly found at the foot of eastern escarpments like FilfilSolomuna, Ela-aro, Mutsub, Ghindae, Anseba River (Daarit), and Halibmentel.

Uses: *Trichiliaemetica* has a wide variety of uses. The skinned seeds are edible. When they are soaked in water or ground, they enhance the flavour of spinach dishes. The oil that is extracted from the seed kernels and husks is excellent for soap and candle making as well as being used as superior furniture oil. The oil is also widely used in cosmetics and as a food preservative. The powdered bark of this remarkable tree is used as an emetic and is effective for the treatment of rheumatism.

METHODOLOGY

Materials and chemicals: Petroleum ether, methanol, distilled water, formalin aldehyde Mueller Hinton agar, SDA (Sabourand dextrose agar), Normal saline, phosphorus buffered saline and Amoxicillin. Materials used Rota vapour, Vacuum pump, and shaker, petri dishes, oven and Auto clave.

Plant collection and authentication: *B.Paprifera, S.singuena*, and *T.emetica* Barks were collected from Halibmentel, Anseba Region-Eritrea, on 12 to 16 of September 2013.The plants were authenticated by Botanist Mr Biniam (MSc) from Department of Biology, Eritrea institute of technology (EIT).

Extraction of crude extract: The extraction method used was cold extraction. Powdered air-dried stem bark of *B.paprifera, S.singuena*, and *T.emetica* was prepared. 100g of each plant bark powdered had macerated using different volume petroleum ether,

methanol, and distilled water of increasing polarity for 48 hour with the help of shaker, and then the extracts were filtered with the help of the vacuum pump. After filtration since the extraction method used was cold extraction the extracts were concentrated in the Rota vapour at 40^{o}c with the help of vacuum pump to yield a dry extract.

Antibacterial test (ABT): Two standard bacteria *S.aureus* (ATCC 10231) Gram positive and *E. coli* (ATCC 25922) Gram negative was procured from Freud hallows IOL laboratory in Asmara. Antibacterial activity was tested by well method with media Muller Hinton agar four times. The bacteria agar Medias were impregnated to the wells with crude extracts of different concentration (100 mg/ml, 50 mg/ml and 25 mg/ml) for each bacteria. 100 micro litres of each crude extract were dropped in the holes in the Muller Hinton agar media for the cultured bacteria. Amoxicillin is used for the positive control in order to compare with the extract results. The culture media incubated at 37°C, for 24hrs Sensitivity test of bacteria was done with the crude extracts. The mean of inhibition zone was measured using a ruler for the crude extracts.

DISCUSSION

The purpose of this research was to demonstrate the traditional practices and uses on these plants for their antibacterial activity in scientifically sound way. So in this research extraction of the three plants *Boswelliapapyrifera, Sennasinguenna and Trichliaemetica* have done using three different solvents for each plant. The solvents were Petroleum ether, Methanol (95%) and Water according to their polarity increment, by using different instruments and chemicals the extraction was processed. At last using the rota vapour it was concentrated and dried at 40^{0}c. The crude dry extract obtained was higher in methanol extract and lower in petroleum ether extracts. In comparing the obtained products according to the polarity of the solvents these plants are richer with polar compounds than nonpolar compounds, water is the most polar one and methanol is a moderate polar which can solubilize both the polar and nonpolar compound that is the reason for the methanol extract to have the highest yield value. For the sensitivity test two micro-organisms have selected which are *E.coli*, and *S.aureus*. The selecting of bacteria was depending on their resistance and their classification as *gram positive (S.aureus)* and *gram negative (E.coli)* bacteria. *E.coli* is one of the highly resistant bacterium and *S.aureus* is less resistant one. Muller Hinton agar has a positive control which is a good media for microbial growth, and also the solvents water, methanol and petroleum ether have no any negative impact on the growth of the selected microbes.

The method used for carrying out of the sensitivity test was well method. This method is preferable for its short time consuming. This type of method is having wells where the crude extract and standard drugs are placed or dropped for diffusing to the media; this method gives a better result than the disc diffusion method. Muller Hinton agar media was the type of the media we have used for the test and it was prepared according the manufacturer instruction at the concentration of 38g/litre. In the antimicrobial test we were trying to work with the aseptic techniques to reduce contamination in the growth media. The growth of bacteria in the incubator was taken place at 37^{0}c.

As we come to the result generally all the plants have shown antimicrobial activity with different strength of concentrations. Methanol extract was predominating in all the antibacterial activity but the petroleum ether extract of all the plants didn't show any zone of inhibition. According to the microbes the *S.aureus* found to be less resistant to the extract of methanol and water. But the *E.coli* is somehow resistant compare to *S.aureus*. Regarding *S.aureus* all the plants are effective with both water and methanol extract. *E.coli* is inhibited by extract of methanol and water of each plant at the highest concentration used in this experiment (i.e.100mg/ml).

The concentrations were prepared at different strengths of 100mg/ml, 50mg/ml, and 25mg/ml. We have applied all the extract concentrations over the *E.coli* and *S.aureus.*

The maximum inhibition zone diameter has been attained by *Sennasinguena*a gainst the microbes in water and methanol extract at all concentrations. But there is no much significant difference between the 100mg/ml and 50mg/ml of *Sennasinguena*. *Trichelliaemetica* has less antibacterial activity than *Sennasinguena and Boswelliapapyrifera, Boswelliapapyrifera* has activity in both water and methanol extract for the selected microorganism.

CONCLUSION

All the selected plants for the study i.e. *Boswelliapapyrifera, Sennasinguena* and *Tricheliaemetica* have antibacterial activity with both extracts of water and methanol. Petroleum ether extracts of all the plants have no any activity against *E.coli*, and *S,aureus.*. *Sennasinguena* is with strong activity against the selected microorganisms. Methanol extract is with the higher antibacterial activity than that of water extracts. There is high yield of crude dried extract in methanol extraction

than in water and very low amount for petroleum ether extract. The compounds responsible for antibacterial activity are extended between the borders of high Polar to moderate polar compounds. The compounds which are dissolved in nonpolar solvent (petroleum ether) from the selected plants have not any antibacterial activity. For all the extract their antibacterial activity is concentration independent.

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Table 1: Crude dry extracts with different solvents from 100g mass of each plant bark

	Solvents					
	Petroleum ether		Methanol (95%)		Distilled water	
Plants	A.S.U	D.C.E	A.S.U	D.C.E	A.S.U	D.C.E
B.papyrifera,	400ml	2.03g	400ml	16.25g	450ml	9.35g
S.singuena	400ml	0.65g	400ml	15.32g	450ml	6.19g
T.emetica	600ml	0.71g	500ml	8.21g	600ml	4.89g

A.S.U= amount of solvent used, D.C.E= dry crude extract

Table 2: Inhibition zone diameter of *B.papyrifera* extracts in mm

		Petroleum ether extract			Methanol (95%) extract			Distilled water extract		
Concentration in mg/ml		*100*	*50*	*25*	*100*	*50*	*25*	*100*	*50*	*25*
Bacteria	*E.coli*	--	--	--	22±0.01	20±0.02	16±00	20±0.01	18±0.03	14±0.03
	S.aureus	--	--	--	32±00	30±0.03	29±0.01	28±0.05	26±00	24±0.04

--: no activity

Results expressed in mean ± standard deviation

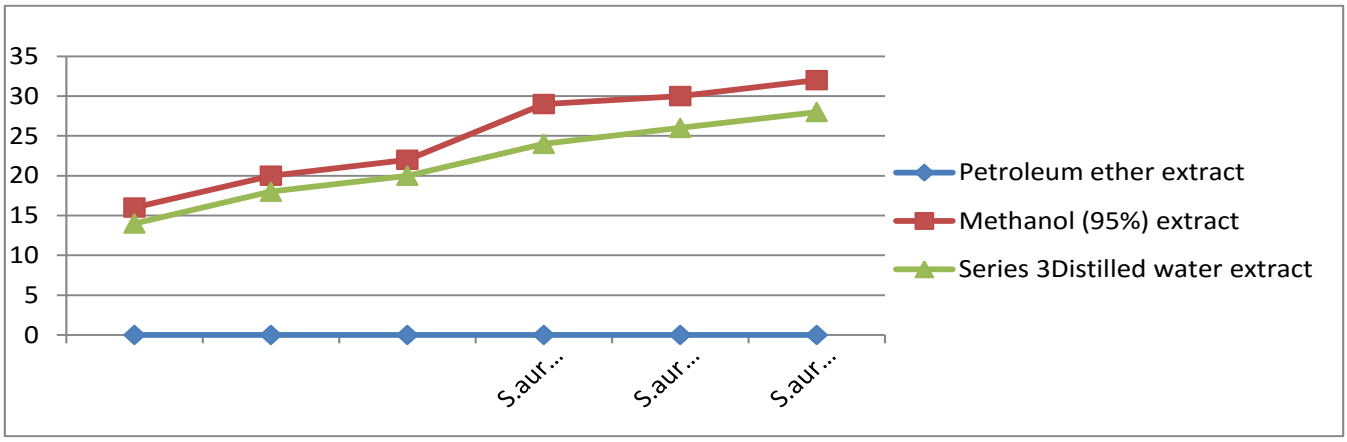

Chart: 1 *B.papyrifera against S.aureus and E.coli*

Table 3: Inhibition zone diameter of *S.singuena* extracts in mm

		Petroleum ether extract			Methanol (95%) extract			Distilled water extract		
Concentration in mg/ml		*100*	*50*	*25*	*100*	*50*	*25*	*100*	*50*	*25*
Bacteria	*E.coli*	--	--	--	28±0.03	26±0.01	22±0.0	22±0.01	21±0.05	18±0.02
	S.aureus	--	--	--	46±0.02	44±0.03	38±0.0	36±0.02	34±0.0	30±0.06

--: no activity

Results expressed in mean ± standard deviation

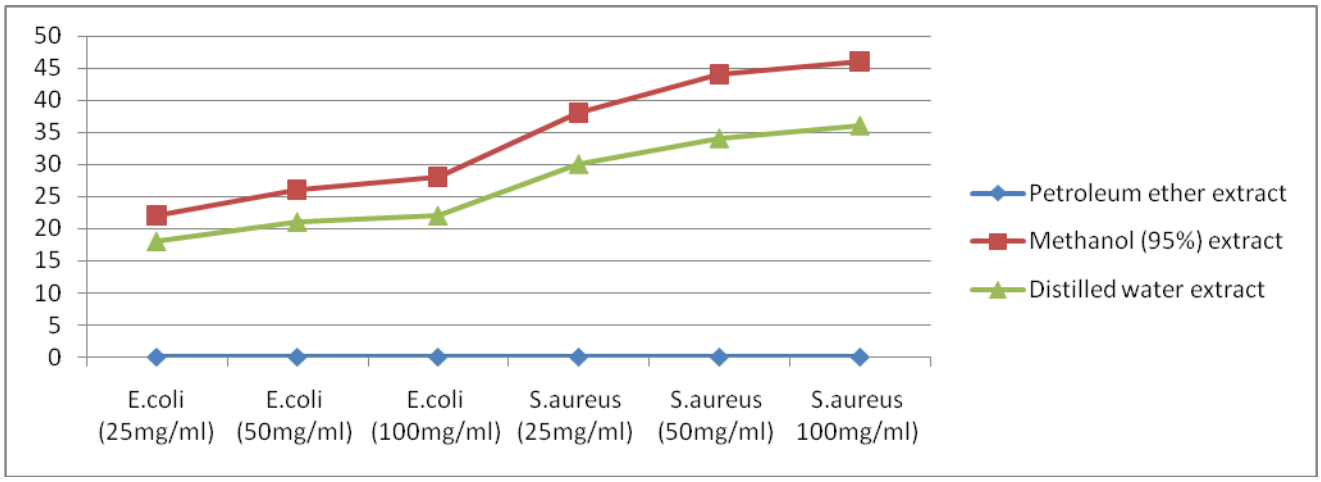

Chart: 2 *S.singuena against S.aureus and E.coli*

Table 4: Inhibition zone diameter of *T.emetica* extracts in mm.

		Petroleum ether extract			Methanol (95%) extract			Distilled water extract		
Concentration in mg/ml		*100*	*50*	*25*	*100*	*50*	*25*	*100*	*50*	*25*
Bacteria	*E.coli*	--	--	--	18±0.05	16±0.05	12±0.04	12±0.01	9±0.04	--
	S.aureus	--	--	--	34±0.0	31±0.03	28±0.06	18±0.04	14±0.0	--

--: no activity
Results expressed in mean ± standard deviation

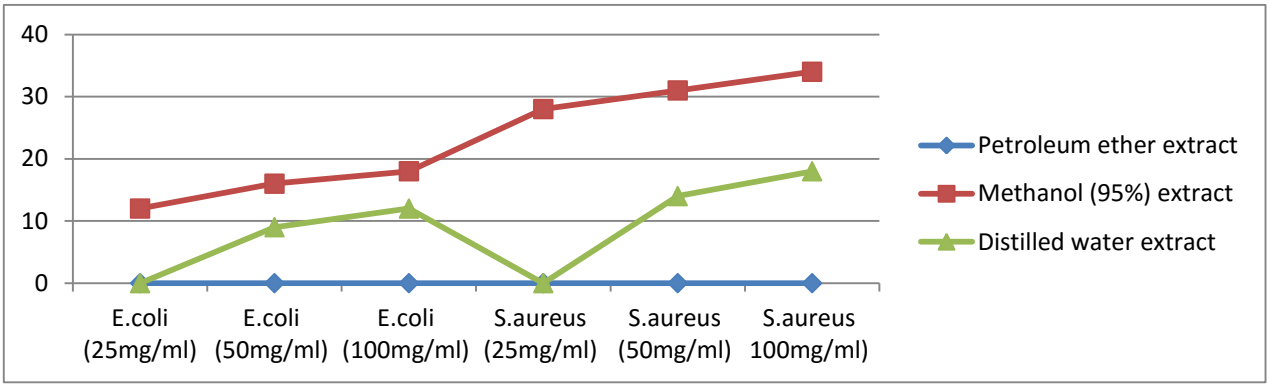

Chart: 3 *T.ematica against S.aureus and E.coli*

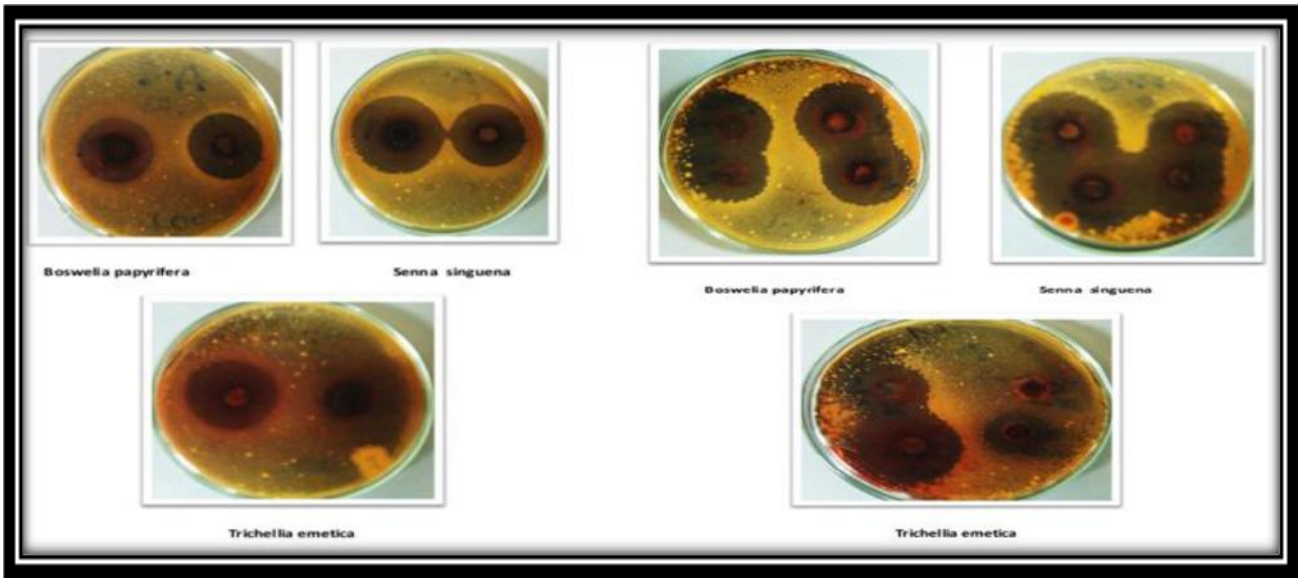

Figure 1 crude extracts against *S.aureus*

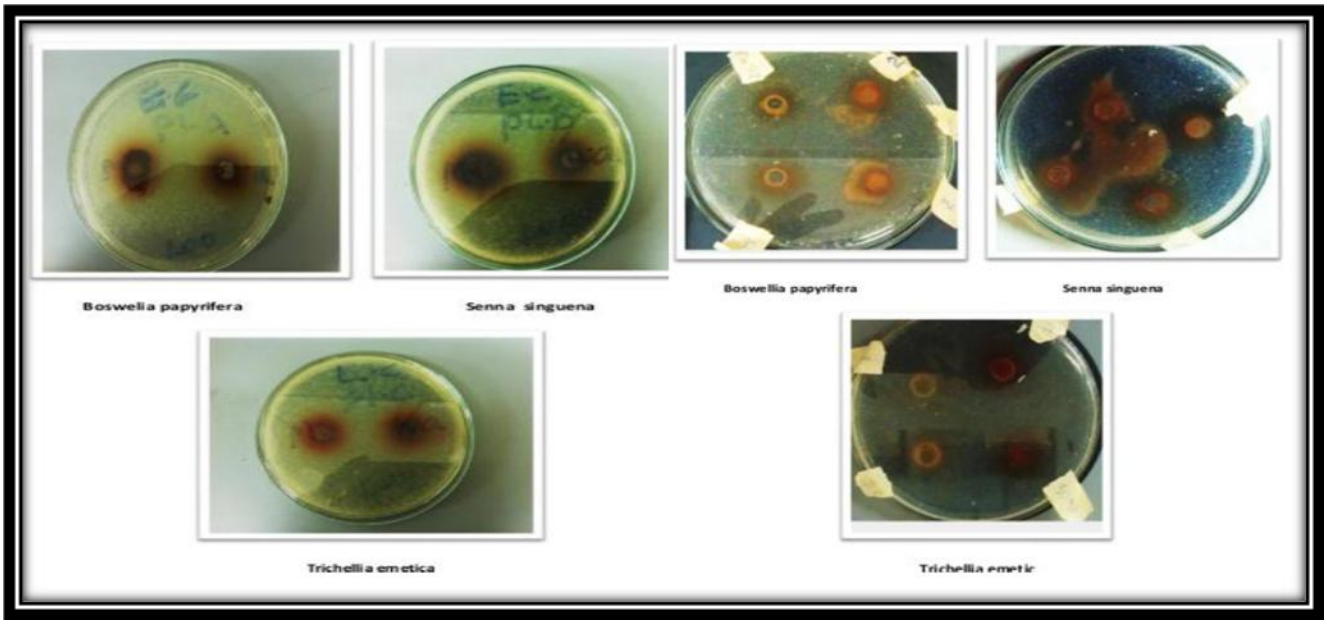

Figure 2 crude extract against *E.coli*

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