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GERMICIDAL EFFICACY OF DIFFERENT LEAF FRACTIONS OF MILKWEED

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ABSTRACT

Gerrmicidal potential of organic fractions of *Calotropis procera* (Ait.) R. Br. (milkweed) leaves was determined by using agar well dissemination technique. Fractions of milkweed leaves were prepared with ethyl acetate, butanol, water (aqueous) and hexane. Among them, fractions with hexane solvent were proved to be the best germicidal agents. It is also concluded that milkweed hexane leaves fraction may be a significant preventive agent against gram negative (*Salmonella typhi* and *Escherichia coli*) as well as gram positive bacteria (*Micrococcus luteus* and *Staphylococcus aureus*).

Keywords: *Calotropis procera*, milkweed, *Staphylococcus aureus*, *Escherichia coli*, Germicidal.

INTRODUCTION

Irresistible ailments are typically constrained by industrially accessible germicidal medications that generally produce side effects in the human body which urge the specialists to find alternate ways to treat illnesses with less or zero poisonous effects (Ali *et al*., 2014). In the previous couple of decades, a few new characteristic germicidal mixes were found for the control of extreme diseases. Revelation of another germicidal specialist against multidrug safe living beings is still going full speed ahead because of the advancement of ceaseless obstruction by organisms. These living beings have gotten incredible clinical consideration on account of expanding revealed cases far and wide. Alongside this, there is an expansion in buyer interest for the medications segregated from common sources (Ali *et al*., 2014). The threat presented to overall population wellbeing by different pathogens can be settled by the disclosure of common germicidal mixes having powerful expansive range restraint against pathogens predominant in the nearby network (Ali *et al*., 2014).

Various workers paid attention to the antibacterial viability of milkweed leaves against bacteria that cause diseases in human beings viz., Neenah *et al*., (2011); Ali *et al*., (2014); Salem *et al*. (2014); Khairnar *et al*., (2012) and Shetty *et al*., (2015). Milkweed is known for its medicinal properties, for example, its leaves possess mitigating and germicidal activity (Basu and Chaudhury, 1991; Neenah *et al*., 2011; Ali *et al*., 2014). Joshi and Kaur (2013), Kawo *et al*. (2009) and Hemalatha *et al*. (2011) reported that different specialists checked the leaf concentrates of *C. procera* in ethanol for its germicidal activity which fundamentally restrained the development of *Bacillus pumilus*, *Proteus vulgaris*, *Staphylococcus aureus*, *Micrococcus luteus*, *Pseudomonas aeroginosa*, *Escherichia coli* and *Bacillus subtilis*. Similarly, Joshi and Kaur (2013) reported the germicidal capability of methanol, ethanol, and fluid concentrate of milkweed and found ethanolic concentrates have strong germicidal action against *P. aeroginosa*. Later, Javadian *et al*. (2014) assessed the germicidal activity of ethanol concentrate of milk weed and found it successful against *E. coli*.

Yesmin *et. al*. (2008) reported the intense germicidal activity of leaves fluid and methanol concentrates of milkweed against Gram +ve and Gram -ve bacteria. Salem *et al*. (2014) also recorded the strong capability of water and ethanolic part against Gram -ve and Gram +ve pathogens. Kareem *et. al.* (2008) uncovered positive activity of chloroform and ethanol parts against *Miocrosporum* sp., *Candida* sp., *Streptococcus pneumoniae*, *E. coli*, *Aespergillus niger*, *S. aureus* and *Streptococcus pyogens*. Kawo *et al*., (2009) contemplated the germicidal activity of ethanol and water leaves concentrate of milkweed and found ethanol extract more effective against bacteria. Further, Goyal and Mathur (2011) tested divisions of ether, butanol and ethanol against *Candida albicans*, *Candida para*, *Pseudomonas aeroginosa*, *Enterococci sp.*, *E. coli* and *Staphylococci* which displayed critical germicidal potential.

Hemalatha *et al*. (2011) revealed inhibitory activity of leaf concentrate of *C. procera* in ethyl acetate, water and methanol against *S. typhi*, *Shigella*, *Pseudomonas aeroginosa*, *Vibrio cholera*, *B. cireus*, *B. subtilis*, *Lactobacillus* and *S. aureus*. Moreover, Johnson *et al*. (2011) verified that fluid and alcoholic concentrate of milkweed leaves shows strong germicidal viability against *S. aureus*, *Aspergillus sp.*, and *E.coli*. While, Velmurugan *et al*. (2012) contemplated milkweed leaf concentrate of ethyl acetate successfully suppressed smaller scale pathogens from shrimp fish. Similarly, Shetty *et. al.* (2015) considered the germicidal impact of ethyl acetate, methanol, derivation, ethanol and water concentrates of milkweed leaves against disease causing bacteria in human and discovered that leaves extricate showed noteworthy antibacterial activity against *M. aureus* in all test solvents. While, Pandey *et. al*. (2015) evaluated the milkweed leaves extract in methanol against *S. aureus* and *E.coli.* He found positive results. Bilal *et. al*. (2020) utilized methanolic extract against various bacteria and found it highly active against *Pseudomonas aeruginosa*, *Bacillus cereus* and *Proteus mirabilis*. Ali *et. al*. (2014) reported the positive action of flower extracts against human pathogenic as well as multidrug resistant bacteria.

The present study was conducted to check the efficacy of *C. procera* extracts in four different solvents i.e. butanol, ethyl acetate, water and hexane against gram –ve and gram +ve positive disease causing bacteria in human. Further to validate the earlier disclosures for its vital use.

MATERIALS AND METHODS

Plant material, concentrate and part course of action: Fresh leaves of milkweed were collected from the vicinity of Karachi. Specimens were deposited to Karachi University Herbarium (KUH) to get the general herbarium number "GH No. 86455". After major washing with water the material was air dried in shade for 3 weeks. To get a grungy concentrate, material was immersed in eighty percent ethanol for ten days and filtered by channel paper. From this concentrate fractions of ("ethyl acetate", "hexane" and "water") were set up by using disengaging channel. The filtrate was then reduced by turning evaporator "Buchi Rotavapor R-200 – Buchi Labortechnik AG, Switzerland". While, division of butanol was united by "Eyela Rotary Vacuum Evaporator Model No. N-10, Tokyo Rikakikai Co. Ltd. Japan". Then sample was secured in low temperature until further use.

Test Microbes and Culture Media: The germicidal activity of milkweed leaf extracts was assessed against G-ve bacteria and Gram +ve bacteria. Gram –ve bacteria i.e. *S. typhi* and *E. coli* ("O157:H7") were isolated from a filthy water test. While, G+ve bacteria i.e. *M. luteus* "KIBGE-IB20" ("GenBank promotion: JQ250612") and methicillin safe *S. aureus* (MRSA) "KIBGE-IB23" ("GenBank increase: KC465400") were secluded from soil test individually. Supplement stock was utilized to re-establish trial strains by hatching at 37^{o}C for 24 hours, with consistent shaking of 135 rpm and were kept up on supplement agar inclines at four degree centigrade for further investigations.

Technique for germicidal activity: To assess the germicidal capability of leaf concentrates of milkweed against germ strains. Agar well dispersion technique was embraced from past studies (Ali *et al*., 2014; Tagg and McGiven 1971; Heatley 1944). Bacterial species were restored on disinfected Petri plates which were loaded with supplement agar and immunized 100µl of pathogenic strains having 10^8 colony forming unit per ml contrasted and the "0.5 McFarland turbidity index" (Ali *et al*., 2014: Iqbal 1998). then 100µl of concentrated leaf evacuates were poured in the agar wells of petri plate and were agonized at 37^{o}C for one day. While, for control no concentrate was poured in the plate. To calculate the potential against germ strains, valid regions were assessed in triplicates with the standard deviation of ± 3.

RESULTS

Leaves extracts of milkweed in different solvents viz., hexane, butanol, water and ethyl acetate were analyzed against different germs.

Different solvents of leaf concentrates of milkweed displayed variety in the restraint range against *Micrococcus luteus*, *Salmonella typhi*, *methicillin -resistant Staphylococcus aureus*, and *Escherichia coli* (Table 1, Fig. 1). Among four concentrates, hexane portion has been found significant as germicidal against every single germ strain. A most extreme region of hindrance (23mm) was seen against *Micrococcus luteus*. While, Ethyl acetate likewise indicated considerable inhibitory action against "MRSA" (15mm) and "*E. coli*" (12mm). Though, "butanol" and "ethyl acetate" did not demonstrated any big action against *M. luteus*. While, no action was seen in butanol and water against "*E. coli*".

Table 1. Germicidal activity of *milkweed* leaf evacuates against different germ strains.

S. No.	Concentrated leaf evacuate 100µl	Valid regions of inhibition (mm)							
		S. typhi	Control	*E. coli*	Control	MRSA	Control	*M. luteus*	Control
1	Butanol	---	---	---	---	7 ± 3	---	---	---
2	Ethyl Acetate	---	---	12 ± 3	---	15 ± 3	---	---	---
3	Aqueous	---	---	---	---	---	---	19 ± 3	---
4	Hexane	15 ± 3	---	18 ± 3	---	12 ± 3	---	23 ± 3	---

Key: Valid region = > 11 mm, --- = No result observed.

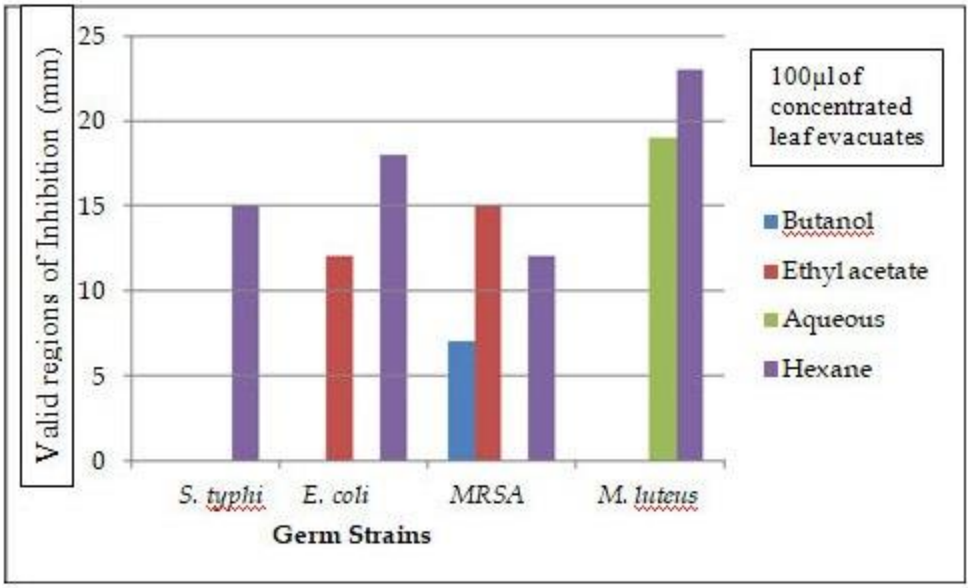

Fig. 1. Valid regions of milkweed leaf derivatives (hexane, butanol, aqueous and ethyl acetate) against germ strains.

DISCUSSIONS

Protection from various expansive range anti-microbial agents has now turned into a worldwide worry because of developing instances of medication resistance which expands buyer request towards natural germicidal operators. So, there is a need of screening of natural germicidal compelling mixes against various medications safe pathogens. Over the most recent decades, a few new common germicidal mixes were found for the control of serious contaminations. Present examination was intended to investigate the germicidal capability of restoratively imperative leaves of milkweed. Distinctive dissolvable concentrates of milkweed indicated unique restraint design against tried illness creating microorganisms. Leaf concentrates of milkweed demonstrated different ranges of restraint against *S. typhi, E. coli, M. luteus* and methicillin resistant *S. aureus* (MRSA) (Table 1., Fig. 1). Among the different concentrates, the milk weed hexane division has been demonstrated huge region as germicidal operator against the examined pathogens. The largest region of hindrance of hexane (23mm) was seen against *M. luteus* when contrasted with butanol and ethyl acetate parts which did not demonstrated any action against *S. typhi* and *M. luteus*. Present results are in agreement with the findings of Ali *et.al.* (2014), who obtained the similar results with flower extract in hexane.

In any case, present finding is in accordance with that of the findings of Doshi *et al*. (2011) where a hindrance region of milkweed leaf extract for *M. luteus* was seen at 9mm. Like the findings of Joshi and Kaur (2013) hexane solvent of leaf separate indicated considerable activity against *E. coli*. On the other hand, ethyl acetate part of leaf additionally indicated critical zone of restraint (12mm) against *E. coli* and *MRSA* (15mm). These readings are conversely with the perception of Doshi *et al*. (2011) where 7mm restraint zone was mentioned. Similarly, butanol concentrate shows considerable (7mm) region of hindrance. Therefore, leaf concentrates of milkweed observed in present study may be referred to as a strong germicidal agent.

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