

Anti-microbial activity of seed oils & protein estimation of seed-content in *Clitoria ternetea*, *Lagenaria siciraria* & *Ziziphus mauritiana*

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Abstract

Asian plants of flower, vegetable and fruit named *Clitoria ternetea*, *Lagenaria siciraria* and *Ziziphus mauritiana* respectively are known for their extensive uses in traditional medicine as well as traditional treatment of various diseases and infections. Several studies have already been done so far on these plants and its various parts but the studies on their seeds as well as their seed oil was lacking. In this work, we determined the protein content of these seeds as well as we extracted the seed oils and determined their anti-microbial activity independently along with the anti-microbial activity by mixing all these oils together in equal ratios. Both, protein determination and anti-microbial activity determination showed considerable and potential results. The *Lagenaria siciraria* seeds (in ethanol extract) showed highest protein content amongst all i.e., 4.44 ($\mu\text{g}/\text{mL}^{-1}$) whereas *Clitoria ternetea* seeds displayed highest protein content (in water extract) amongst all i.e., 5.07 ($\mu\text{g}/\text{mL}^{-1}$). The anti-microbial activity of the mixed seed oil showed better and great results as compared to individual anti-microbial activity of seed oils.

Keywords: *Clitoria* genus, *Lagenaria* genus, *Ziziphus* genus, seed oil, biological activity.

Atividade antimicrobiana de óleos de sementes e estimativa do teor de proteína de sementes em *Clitoria ternetea*, *Lagenaria siciraria* e *Ziziphus mauritiana*

Resumo

Plantas asiáticas de flores, vegetais por frutas chamadas *Clitoria ternetea*, *Lagenaria siciraria* e *Ziziphus mauritiana*, respectivamente, são conhecidas por seus usos extensivos na medicina tradicional, bem como no tratamento tradicional de várias doenças e infecções. Vários estudos já foram feitos até agora sobre essas plantas e suas várias partes, mas faltavam estudos sobre suas sementes, bem como seu óleo de semente. Neste trabalho, determinamos o teor de proteína dessas sementes, bem como extraímos os óleos das sementes e determinamos sua atividade antimicrobiana independentemente juntamente com a atividade antimicrobiana, misturando todos esses óleos em proporções iguais. Tanto a determinação de proteínas quanto a determinação da atividade antimicrobiana mostraram resultados consideráveis e potenciais. As sementes de *Lagenaria siciraria* (em extrato etanólico) apresentaram o maior teor de proteína entre todas, ou seja, 4,44 ($\mu\text{g}/\text{mL}^{-1}$), enquanto as sementes de *Clitoria ternetea* apresentaram o maior teor de proteína (em extrato aquoso) entre todas, ou seja, 5,07 ($\mu\text{g}/\text{mL}^{-1}$). A atividade antimicrobiana do óleo da semente mista apresentou melhores e ótimos resultados em comparação com a atividade antimicrobiana individual dos óleos de semente.

Palavras-chave: gênero *Clitoria*, gênero *Lagenaria*, gênero *Ziziphus*, óleo de semente, atividade biológica.

1. Introduction

Plants derived natural and herbal medicines forms strongest and main component in Asian treatment. In here, we took seed samples of different plants that are consumed directly or indirectly on a wide scale without knowing

their several hidden benefits. *Clitoria ternatea* (*C. ternatea*) which is commonly known as “Aparajita” in India; is also addressed by several other names such as butterfly pea, shankha pushpa, bungatelang, blue pea, cordofan pea, blue-bell vine and pigeonwings. It is a flowering plant which is native to Southeast Asia whose fruit is 5-7 cm long flat pods containing 6-10 seeds per pod; these pods are narrow and contains small; black seeds. These seeds are edible when fresh and tender. Seeds are good stock food hence, young pods are edible and used as vegetables.

All parts of *C. ternatea* contain peptides called cliotides which shows potent anti-microbial properties against *Escherichia coli*. The seeds of *C. ternatea* are protein-rich (Bravo, 1971; Odeyinka et al., 2004; Schlink et al., 1993); also are a strong purgative (Cook et al., 2005) and contains both condensed tannins and trypsin inhibitors (Bravo, 1971; Macedo et al., 1992). The seeds of this plant have great medicinal properties; thus it helps in detoxify stomach and other body organs, improves digestion, ease stomach ache, reduces inflammation, stimulates hair growth and much more.

The *Lagenaria siceraria* (*L. siceraria*) is a member of the Cucurbitaceae (squash) family which is commonly known as “Bottle gourd”. Its other common names are calabash, dipper gourd, birdhouse gourd, white-flowered gourd and long melon. This climbing perennial plant is extensively grown as a vegetable crop in tropical regions such as India, Thailand, China, Japan, and the rest of the world (Hussein et al., 2021; Upananlawar, 2017) and is used in many Asian households in cooking as a vegetable. The fruits of Bottle gourd come in variety of shapes like; huge and rounded, small with bottle shape or slim and serpentine and also they can potentially grow over a meter long. The round varieties are typically known as calabash gourds. These gourds contain seeds inside the fruit that are rich in various minerals such as Calcium, Iron, Potassium, Magnesium, Zinc, Lead, Sodium, and Chromium (Ojiaka; Igwe, 2007). Not only this but these seeds also contains seventeen amino acids namely; leucine, lysine, isoleucine, histidine, cysteine, methionine, tyrosine, threonine, proline, glutamic acid, phenylalanine, arginine, valine, serine, alanine, glycine, and aspartic acid (Hassan et al., 2008). Thus, these seeds are a valuable source of proteins, lipids, micro and macro-nutrients.

Ziziphus mauritiana (*Z. mauritiana*) or “Indian jujube” originated from Central Asia and then got spread to regions of North Africa and India through Afghanistan, Malaysia, China and parts of Australia. Indian jujube can be grown in semi-arid as well as in arid regions as it can thrive under very dry conditions. Whole plant of *Z. mauritiana* including fruits, seeds and leaves are valued for their incredible healing characteristics. It is a good source of carotene, vitamins A and C, and fatty oils (Orwa et al., 2009) and its seeds are used for medicinal purposes in traditional Chinese and Indian medicine. It may also possess anxiety reducing properties, sedative properties and is most often used to treat mental health and digestion related issues. Presence of ample proteins, minerals and vitamins in jujube seed essential oil are known to possess hair growing properties. Jujube seed oil when mixed with carrier oil applied regularly to stimulate faster hair growth and prevents hair loss.

Taking protein supplements that are available from natural resources is beneficial and proposes good health with no side effects or harm when compared to synthetically/artificially available supplements available in the market. All the three samples used here have high protein content and thus can be considered to sustain a good and healthy lifestyle.

Antimicrobial activity can be defined and described as a collective term for all active agents that acts by inhibiting the growth of bacteria, prevents the formation of microbial colonies and can also destroy micro-organisms. Antibacterial activity has remained the most important characteristic in medical industry that provides adequate protection against micro-organisms, biological fluids, aerosols as well as in disease transmission. Antimicrobial agents must have three essential characteristics in order to showcase their full potential for effective therapy in critically ill patients; namely in vivo and in-vitro effect, lack toxicity and must have reasonable cost. It's been proved in many studies that seeds are and can be the potential antimicrobial agents. Taking easy cultivable and accessible seeds and extract their oils which can be used as antimicrobial source along with this these oils will provide nutrients and moisture to the skin wherever applied.

These analysis were performed to check the potential of few seeds in order to provide a natural protein supplement (powdered form) and a natural treatment in the form of oil for various skin infections due to varied range of bacteria's and fungi including scalp infections, promote hair growth, reduce hair loss and helps delay greying of hair.

2. Materials and Methods

2.1 Sample preparation

The seeds of *C. ternetea*, *L. siciraria* and *Z. mauritiana* were collected and air dried for the protein estimation whereas some amounts of these seeds were dried in hot air oven at 50 °C for 6 hours for the removal of moisture in order to proceed with oil extraction process. Both the air and oven dried seeds were crushed and grinded into fine powder using mortar and pestle for protein estimation and oil extraction.

2.2 Determination of proteins

The prepared seeds powder were weighed 5 g each and were dissolved separately in 30 mL ethanol and 30 mL distilled water. Now, *Folin Lowry* reagent was prepared and using this FC reagent protein estimation was carried out in which 5 standards and blank were also prepared. Each sample was suspended both in 30 mL ethanol and 30 mL water and these suspension were left at room temperature; overnight. The following day the prepared suspension mixtures were filtered and were ready for protein estimation using FC reagent whose optical density was recorded at 670 nm.



Figure 1. From left to right: *Lagenaria siciraria* (C₂H₅OH), *Ziziphus mauritiana* (H₂O), *Ziziphus mauritiana* (C₂H₅OH), *Clitoria ternetea* (C₂H₅OH), *Clitoria ternetea* (H₂O), *Lagenaria siciraria* (H₂O) prepared samples for protein estimation. Source: Authors, 2023.

2.3 Oil extraction

Seed powders of *C. ternetea*, *L. siciraria* and *Z. mauritiana* were weighted 20 g, 80 g and 80 g respectively and were soaked in 30 mL, 100 mL and 100 mL respectively in *n*-hexane overnight. The prepared suspension mixtures were filtered and subjected to oil extraction process using *Soxhlet* apparatus. The oil extraction process for all three mixtures was performed separately.

2.4 Determination of anti-microbial activity

The microbial activity against gram +ve bacteria *Staphylococcus* spp. and gram –ve bacteria *Escherichia coli* was studied using Agar Well Diffusion method. We prepared fresh nutrient agar plates and wells were punctured in each plate. We took individual samples of oils as well as a mix of oil was prepared in which equal ratios of oils were mixed. These oil samples were poured 30 µL each in the separate wells using micropipette; in mixed oil sample we took 10 µL of each oil sample and the colonies of gram +ve *Staphylococcus* spp. bacteria and gram –ve *Escherichia coli* bacteria were streaked in each plate, the plate was then incubated for 24 h at 37 °C. The results were expressed in millimeters (mm) of inhibition, using a digital caliper.

3. Results and Discussion

3.1 Determination of proteins

The prepared samples of powdered seeds in both the medium ethanol and water upon filtration were reacted with FC reagent and the optical density of each sample was recorded using spectrophotometer at 670 nm. The protein content of each sample was observed as; *C. ternetea* (in ethanol) 1.68 µg/mL⁻¹, *L. siciraria* (in ethanol) 4.44 µg/mL⁻¹ and *Z. mauritiana* (in ethanol) 3.55 µg/mL⁻¹. Whereas, the water samples of each of these showed following protein content; *C. ternetea* had 5.07 µg/mL⁻¹, *L. siciraria* had 1.95 µg/mL⁻¹ and *Z. mauritiana* with 4.05 µg/mL⁻¹.

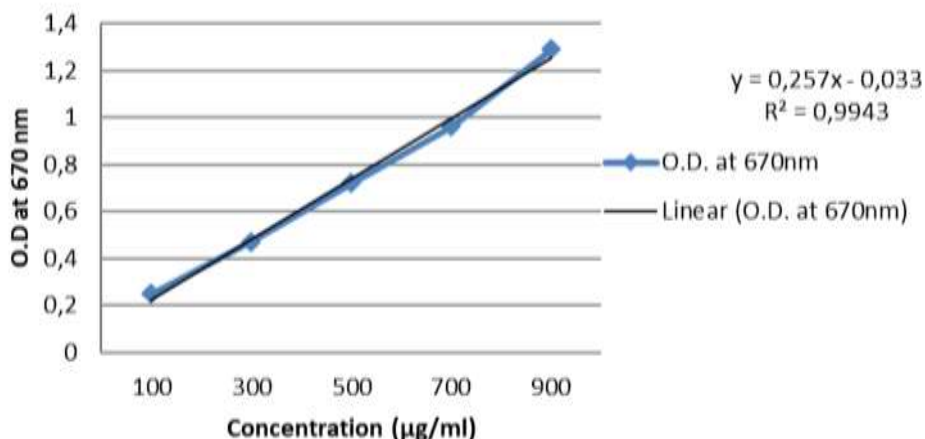


Figure 2. Standard graphical representation of protein estimation. Source: Authors, 2023.

3.2 Oil extraction

The oil extraction process was conducted for each sample separately. Here, we used *n*-hexane as a solvent. It was found that *L. siceraria* showed high yield in comparison to *Z. mauritiana*. On the other hand where we took *C. ternetea* showed unexpected amount of yield from very less sample when compared to both *L. siceraria* and *Z. mauritiana*.



Figure 3. From left to right: *Clitoria ternetea*, *Lagenaria siceraria* and *Ziziphus mauritiana* extracted seed oil in respectively order in picture. Source: Authors, 2023.

3.3 Determination of anti-microbial activity

The anti-microbial activities of individual seed oils along with a mixture of these oils were tested. We took prepared Nutrient Agar plates and using Agar Well Diffusion method the anti-microbial activity of each sample was conducted. All the samples displayed anti-microbial activity, but high anti-microbial activity was observed with mixed oil sample with inhibition zone of 10.4 mm against *Staphylococcus* spp. and 7.1 mm against *Escherichia coli*. Thus, we can say that the mixed seed oil of these samples can prove to be a gift of nature to various skin and hair problems.

Among the samples prepared using ethanol as solvent *L. siceraria* showed high protein concentration of $4.44 \mu\text{g}/\text{mL}^{-1}$ and *C. ternetea* sample in water showed high protein content of about $5.07 \mu\text{g}/\text{mL}^{-1}$. In order to get benefits of all the three protein sources it is required to extract proteins from these and modify into powder and then can be introduced in a capsule form with slight formulations as all these three proteins can be beneficial for health and comes from a good natural source.

Studies showed that *L. siceraria* oil has several applications in various industries (Hassan et al., 2008; Piccirilli

et al., 2007; Habibur, 2003). This seed oil was clear and had pale yellow colour (Kubde et al., 2010). According to a researcher *L. siceraria* seeds nearly had 39.22% fatty acid content (El-dengawy et al., 2001); also the biological value of its protein content is about 74.20%. This oil can be a solution to range of concerns such as acne, hyper-seborrhoea, alopecia and hirsutism. The seed oil of *L. siceraria* is also considered to have anthelmintic properties and is used externally for headache. (Piccirilli et al., 2007). The *Z. mauritiana* showed decent anti-microbial activity. A study also showed that this seed oil has anti-inflammatory activity (Sharif et al., 2009). Thus, the seed oil can be used in treatment of various skin inflammatory diseases and infections. Whereas, *C. ternetea* is known for its various medicinal qualities and it is not only rich in protein but also its seed oil is beneficial for hair growth.

4. Conclusions

In this present work, *Clitoria ternetea*, *Lagenaria siceraria* and *Ziziphus mauritiana* seeds were studied for their protein content and also to prove and confirm the antimicrobial properties of their seed oils. These days being physically fit and having gym body has become a new trend and brought competition among people; this made markets flood with super-artificially prepared synthetic protein supplements. To encounter these supplements by natural and a healthy option the seeds derived proteins can be a good choice.

We hereby, determined the protein content in seeds of *C. ternetea*, *L. siceraria* and *Z. mauritiana* and found a good amount in each one of these. Their proteins should be made available mixed together in proper ratio; in powdered form in capsules which can surely eliminate medical ailments and relieve distress. Not only this, but the oils prepared from their seeds showed antimicrobial activity. Thus, using these oil can be very helpful for various skin infections and when mixed in decent proportion this mixed oil showed high quality result and can be a single answer to multiple problems like scalp infections, hair fall, greying of hair, microbial skin infections and dry skin.

5. Acknowledgments

Not applicable.

6. Authors' Contributions

Porshia Sharma: designed & performed analysis, wrote the paper. *Pragya Rathore*: Supervised the project. *Yogesh Choudhary*: collected data. *Yash Tatwade*: analysis tools. *Akshada Joshi*: analysis tools.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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