Evaluation of the phytochemical composition of some commonly sold male herbal fertility supplements in Port Harcourt, Rivers State.

Abstract

Plants are major sources used in the production of pharmaceutically active drugs and they contain diverse phytochemicals which give these herbal supplements their biological and pharmacological effects. This study evaluated the phytochemical constituents of three different herbal male fertility supplements (Libron Herbal, Mascum Herbal Pride and Energy 3000) commonly used in Port Harcourt. The qualitative and quantitative analysis of the herbal supplements was done using standard procedures. The GC 789OB 5977A and Mass Spectrometry Device (MSD) Model was used for the GC/MS Study. Results of the qualitative phytochemical analysis of the supplements shows that flavonoid, protodiocin, tannins, phenols and alkaloids were present in libron herbal supplement. Protodiocin, saponins, tannins, phenols, alkaloids and cardiacglycosides were present in mascum herbal supplement while flavonoids, protodiocin, tannins, alkaloids, anthroquinones and terpeniods were present in Energy 3000 herbal supplement. For the quantitative phytochemical analysis of the herbal supplements, libron herbal supplement contained Flavonoid 0.24 ± 0.00 %, protodiocin 0.29 ± 0.01 %, tannins 0.37 ± 0.02 %, phenols 0.18 ± 0.00 % and alkaloid 0.28 ± 0.10 %. Mascum herbal supplement were seen to contain protodiocin 0.27 ± 0.07 %, saponins 0.48 ± 0.08 %, tannis 0.38 ± 0.06 %, phenols 0.21± 0.07%, alkaloids 0.18 ± 0.10 %, and cardiaglycosides 0.27 ± 0.07 % while Energy 3000 was made up of flavonoid 0.10 ± 0.00 %, protodiocin 0.29 ± 0.04 %, tannis 0.64 ± 0.10 %, phenols 0.28 ± 0.01 %, alkaloids 0.26 ± 0.01 %, anthroquinones 0.27 ± 0.10 % and terpenoids 0.25 ± 0.00%.The GC/MS study was seen to contain various chemical compunds. The three herbal supplements assayed contain diverse phytochemicals that may enhance male fertility as well as some chemical compounds that may alter hormonal functioning of some biochemical systems in accumulated in accumulated quantities.

1. INTRODUCTION

Plants are major sources used in the production of pharmaceutically active drugs by pharmaceutical industries and a very important branch of medicine; as such this active components are being employed in the production of herbal supplements with a general belief that the raw form which is used in the production of herbal supplements are more potent. They have been proven to possess therapeutic potentials, and are widely used by traditionalists in the treatment of various diseases since antiquity (Umeaku *et al.,* 2018).

Plants contain diverse phytochemicals and have been in use by humans from ancient time in many parts of the world including Nigeria for the treatment and prevention of various illnesses (Saxena *et al.,* 2013). Even in the emergence of complementary medicine, herbs are considered one of the most important branches of traditional medicine and as such they play important roles in health care system especially in rural areas and developing countries due to the level of poverty in this part of the world. About 80% of the world's population depends on herbal medicines as their primary source of health care (Bodeker *et al.,* 2005). Herbs and other natural products represent 50% of all currently utilized medication worldwide (Kunle *et al.,* 2012).

The use of plant products in pharmaceutical industries has given herbal product a place beside complementary medicine. Herbal substance is considered medicinal when it possesses the ability to treat or prevent disease (Nasri, *et al.*, 2014). Due to the global usage of herbal supplement, the market is infiltrated with many new products as such public health issues and concerns about its safety demands the knowlwedge of its components as many of them remain untested and unmonitored in the open market. Hence the need to evaluate its phytochemical components. Phytochemicals in plant give them their biological and pharmacological effect in the treatment of various illnesses and also play many roles contributing to the plant colour, texture, aroma, and flavour and also protect the plant cells from environmental hazards such as drought, ultraviolet exposure, pathogens and environmental pollution and also protects humans that eat it in significant amount (Salisu & Nura, 2022). Commonly used herbal supplements in this study were libron herbal supplement composed of *Allum sativum* (Garlic), *Panex ginseng* (Red Ginseng) *Nymbaea albu* (waterlily), *Rosa gallica* (French rose) *Monarda didyma* (Bee Balm) and *Aframomum melegueta*. Mascum herbal pride composed of *Hepo createa pollens*, (Pollen) *Xylopia aethiopica*, (Guinea pepper) *Medicago sativa* (Alfafa or Lucerne) *Tetrapleura tereaptera*, (Aidan fruit) *Urtica dioca*. (Stinging nettle) and Energy 3000 supplement made up of *Citrus aurantifolia* (Lime) (0.5%), *Psidium guajava* (Guava) (0.5%), *Xylopia aethiopica* (Guinea pepper) (1%), *Sesamum indicum* (Sesame) (1%), *Magnifera indica* (Mango) (2%) and water.

2.1 Materials and Methods

Phytochemical screening of all the compounds were carried out using standard method by Trease & Evans, 1989. A Spectro-UV-Visible 2500 was used for the quantitative phytochemical analysis while a GC 789OB 5977A and Mass Spectrometry Device (MSD) Model was used for the GC/MS Study.

2.8 Quantitative Phytochemical Analysis of the Herbal Supplements (Libron, Mascum and Energy 3000)

The following herbal products (Libron capsule and Mascum herbal pride) were properly washed with deionized water to remove dirt. They were all dried under room temperature and pulverized using warring blender.The extraction was done using Soxhlet extractor in a continuous extraction Process for 72 hours using Methanol as the extracting solvent. A weighed portion of the pulverized samples (200 g) was used against 1000 ml of absolute methanol (BDH Chemicals) for

72 hours.The extracts including Energy 3000 were concentrated using rotary evaporator set at 60 ^{0}C. The extract was then prepared using distilled water for quantitative phytochemical analysis. The extract was read using spectrophotometric methods by Spectro-UV-Visible 2500, manufactured by Labo Med Inc., USA via scanning method at different wave lengths in accordance with ISO 17025.

The absorbance obtained was compared with phytochemical reference standard and the results indicated.

2.9. Gas Chromatography and Mass Spectrometry (GC/MS)

The GC/MS study of the herbal drugs was done using a GC 789OB 5977A and Mass Spectrometry Device (MSD) Model. Gas chromatography-mass spectrometry (GC-MS) is a quick and accurate approach for examining the constituents in complicated mixtures. It enables the identification of several chemicals in modest amounts of plant materials. It provides the structure and weight of phytocompounds present in a plant sample (Keke *et al.*, 2023).

3. RESULTS

3.1 Qualitative Phytochemical Test Results of Libron herbal.

The result of the qualitative phytochemical analysis of libron herbal shows that it contains various chemical constituents such as flavonoid, protodiocin, tannis, phenols and alkaloids. They were present in large amount as shown in table 1.

Table 1. Qualitative Screening result for Phytochemical Components in Libron Herbal

Phytochemicals	Result
Flavonoid	+
Protodiocin	++
Saponins	-
Tannins	++
Phenols	+
Alkaloids	+
Terpenes	-
Cardiaglycosides	-
Quinones	-
Steroids	-
Terpenoids	

+ =present
- = absent

3.2. Qualitative Phytochemical Test Results of Mascum herbal pride.

The result of the qualitative phytochemical analysis of mascum herbal pride is shown to contain various chemical constituents, including Protodiocin, saponins, phenols, alkaloids and cardiaglycosides in large quantity as shown in table 2.

Table 2. Qualitative Screening result for phytochemical components in Mascum Herbal Pride

Phytochemicals	Results
Flavonoid	-
Protodiocin	+
Saponin	++
Tannins	++
Phenols	+
Alkaloids	+
Terpenes	-
Cardiaglycosides	+
Quinones	-
Steroids	-
Terpenoids	-

+ = present
- = absent

3.3. Qualitative Phytochemical Test Results of Energy 3000.

The result of the qualitative phytochemical analysis of Energy 3000 is shown to contain various chemical constituents such as flavonoids, protodiocin, tannis, phenols, alkaloids, anthroquinones and terpenoids. These were present in large quantity as shown in table 3.

Table 3. Qualitative Screening result for phytochemical components in Energy 3000

Phytochemicals	**Results**
Flavonoid	+
Protodiocin	++
Saponins	-
Tannins	++
Phenols	+
Alkaloids	+
Terpenes	-
Cardiaglycosides	_
Anthroquinones	+
Steroids	-
Terpenoids	+

+ = present
- = absent

3.4. Quantitative Phytochemical Test Results of Libron herbal.

The results of the quantitative phytochemical analysis of Libron herbal shows that it contains flavonoid 0.24 ± 0.00 %, protodiocin 0.29 ± 0.01 %, tannins 0.37 ± 0.02 %, phenols 0.18 ± 0.00 % and alkaloid 0.28 ± 0.10 % as seen on table 4.

3.5. Quantitative Results of the phytochemical analysis of Mascum herbal pride.

The result of the quantitative phytochemical analysis of Mascum herbal pride shows that it contains protodiocin 0.27 ± 0.07 %, saponins 0.48 ± 0.08 %, tannins 0.38 ± 0.06 %, phenols 0.21± 0.07%, alkaloids 0.18 ± 0.10 %, and cardiaglycosides 0.27 ± 0.07 % as shown on table 5.

3.6. Quantitative Results of the phytochemical analysis of Energy 3000.

The result of the quantitative phytochemical analysis of Energy 3000 shows that it contains flavonoid 0.10 ± 0.00 %, protodiocin 0.29 ± 0.04 %, tannins 0.64 ± 0.10 %, phenols 0.28 ± 0.01 %, alkaloids 0.26 ± 0.01 %, anthroquinones 0.27 ± 0.10 % and terpenoids 0.25 ± 0.00 % as shown on table Table 6.

Table 4. Quantitative Screening result for Phytochemical Constituents in Libron Herbal

Phytochemicals	**Percentage (%) constituents in Libron Herbal**
Flavonoid	0.24 ± 0.00
Protodiocin	0.29 ± 0.01
Tannins	0.37 ± 0.02
Phenols	0.18 ± 0.00
Alkaloid	0.28 ± 0.1

Table 5. Quantitative Screening result for phytochemical Constituents in Mascum Herbal

Phytochemical	Percentage (%) constituents in Mascum Herbal
Protodiocin	0.23 ± 0.10
Saponins	0.48 ± 0.08
Tannins	0.38 ± 0.06
Phenols	0.21 ± 0.07
Alkaloids	0.18 ± 0.10
Cardiaglycosides	0.27 ± 0.07

Table 6. Quantitative Screening result for phytochemical Constituents in Energy 3000

Phytochemical	Percentage (%) constituents in Energy 3000
Flavonoid	0.10 ± 0.00
Protodiocin	0.29± 0.04
Tannins	0.64 ± 0.10
Phenols	0.28 ± 0.01
Alkaloids	0.26 ± 0.01
Anthroquinones	0.27 ± 0.10
Terpenoids	0.25 ± 0.00

Qualitative Results of Gas Chromatographic and Mass Spectrometry of the three supplements.

3.7. Qualitative Results of Gas Chromatographic and Mass Spectrometry of Libron Herbal.

The result of the qualitative Gas chromatographic and mass spectrometry result of Libron capsule shows that it contains various chemical compounds. Cyclohexanol, 1, 5-cyclooctadiene, carvone, 2-cyclohexene-1-ol, beta-famesene, beta capaene, bicyclogermacrene, cyclohexane, deodecanoic acid, carotol, Apoil, methyl tetradecanoate, hexadecanoic acid, 9, 12-octadecadienoic acid, methyl stearate, 4,4,8-trimethyl-non-5-enal and 9,octadecenoic acid.

3.8. Qualitative Results of Gas Chromatographic and Mass Spectrometry of Mascum Herbal.

The result of the qualitative gas chromatographic and mass spectrometry analysis of Mascum herbal pride shows that it contains a large number of chemical constituents. Salicyclic acid, methyl- 8-methyl decanoate, nonanoic acid, benzoic acid, carvone, benzene, cyclohexasiloxane, decanoic acid, cyclo heptasiloxane, dodecanoic acid, 2,5-dihydoxybenzoic acid, methyl tetra decanoate, hexadecanoic acid, 9-octadecanoic acid, methyl stearate, mercaptoacetic acid, diisoocytyl phthate, cis-vaccenic acid and oleic acid is seen to be present in large quantity.

3.9. Qualitative results of Gas Chromatographic and Mass Spectrometry of Energy 3000.

The result of the qualitative gas chromatography and mass spectrometry analysis of Energy 3000 shows that it contains numerous chemical compounds. Endo-Borneol, cis-dihydrocarvone, cyclohexanol, benzoic acid, carvone, cyclohexasiloxane, decanoic acid, trans-carveyl acetate, benzene, cyclopenta cis-beta-farnesene, cis-muurola-4-5-diene, cycloheptasiloxane, gamma-muurolene, Napthalene, bicycle-5-2 non-1-ene, carotol, epicubenol, Apoil, dihydroxybenzoic acid, alpha-cadinol, methyl tetradecanoate, hexadecanoic acid, 9, 12 octadecadienoic acid,methyl stearate, 1,3-octadecenal, oleic acid and 1-5-9-undecatriene.

4. Discussion

The qualitative and quantitative phytochemical components present in Libron herbal tablets, Mascum herbal and Energy 3000 herbal supplements were demonstrated in this study. Several plants have been proven to contain various phytochemicals with these compounds playing essential roles. Miyoung *et al.* (2013); and Kahkonen *et al.* (1999) discovered that phytochemicals in plants serve as antioxidants, antibacterial, antiviral agents as well as possessing anti-inflammatory activities because they act as free radical scavengers as they are potential reducing agents protecting organs from oxidative damage due to the presence of hydroxyl groups. In this study we see that these herbal supplements contain numerous phytochemicals such as flavonoids, anthocyanins and phenolic acid as such they are able to serve as good antioxidants possesing anti-inflammatory activities. Somayeh *et al.* (2018) revealed that *Rosa gallica* (French rose) a component of Libron herbal supplement possesses flavonoids, anthocyanins and phenolic acid thereby exerting its anti-inflammatory effects on the seminiferous tubules. This is similar to studies by Muhammad *et al.*, 2019 in which *Allum sativa* (Garlic) another component of libron was seen to contain flavonoids, saponins, tannins, phenols, alkaloids and terpenoids. Though another study by Ebomoyi & Ahumibe, (2014) gives contradicting results.

Protodioscin are bioactive components and have therapeutic role as such increases the concentration of androgen receptors in cells, thereby causing the organism to become more sensitive to androgens like testosterone as seen in a study carried out by Lakshmi *et al.* (2023). This study reveals the presence of protodioscin in all the three herbal supplement hence we should expect an improvement in testosterone levels upon its usage.

For Mascum herbal, it demonstrates qualitative and quantitative phytochemical components. Shoushtari *et al.* (2018) discovered that Hepo-createa pollens (Pollen) one of its component contains flavonoid, phenols and saponins and exert anti-inflammatory and anti-oxidative effect. Studies by Abedi *et al.* (2012) also reveals that extract of pollen significantly increased sperm motility, LH, FSH and testosterone levels. *Xylopia aethiopica* (Guinea pepper) another component of mascum herbal pride is made up of flavonoids, tannins, phlobotannins, phenol, anthroquinones and saponins and reduces sperm count, motility, testosterone, FSH and LH levels in a study by Yusuf *et al.* (2018).

Ali *et al.* (2021) in his study reveals that *Medicago sativa* (Alfafa or Lucerne), another component of mascum herbal supplement contains saponins, lignin, tannins, alkaloids, triterpenes, glyosides, flavons and sterols as such serves as anti-inflammatory and antioxidant agents. In this study we see that mascum herbal is composed of various phytochemicals as seen in the composition of its various component. Also *Tetrapleura tetraptera* (Aidan fruit), another component in mascum herbal supplement also contains tannins, flavonoid, saponin, triterpenoid and coumarin thereby serving as anti-inflammatory, anti-coagulant and anti-oxidant agents in a study by Adusei *et al.* (2019). Adelakun *et al.* (2021) in his study reveals that this plant improves spermatogenic activity and maintains testicular integrity. In this study we see that mascum herbal contains diverse plants with its phytochemicals as such should be able to increases testicular function.

Okam *et al.* (2016) in his study looked at the effect of sub-chronic administration of Mascum herbal pride on male albino rats and recommended a phytochemical screening in further studies. This study has been able to look at the phytochemical analysis of Mascum herbal.

For Energy 3000, it components, *Citrus aurantifolia* (Lime), contains flavonoids, flavons, triterpenoid which serves as anti-inflammatory, antioxidants and antibacterial thus protecting against bone marrow toxicity caused by lead acetate and also protects the liver and kidney tissue in a study by Aprioku & Obianime (2014). Idris *et al.* (2018) has a contrary result in which *Citrus aurantifolia* (Lime), causes testicular damamge and azoospermia.

Psidium guajava (Guava) another component of Energy 3000 contains large number of antioxidants that gives it its antispasmodic, anti-inflammatory, antimicrobial effect in a study by Oluwole *et al.* (2007). Also Neeta *et al.* (2015) reveals that *Sesamum indicum* (Sesame) another component of Energy 3000 contain flavonoids, phenolic acid, alkaloids, tannins, saponins, steroids and terpenoids this gives it its anti-inflammatory and antioxidant effects.

Jing *et al.* (2018) reveals that *Magnifera indica* (Mango), another component of Energy 3000, contains tannins, polyphenols, flavonoids and triterpenoids as such it serves as a powerful antioxidant. Energy 3000 therefore should posses antiinflammatory and antioxidant effect since it posseses these various phytochemicals.

Apart from the phytochemical studies carried out, GC/MS studies is fundamental in understanding the response that may be seen in different organs as the individual herbal

supplement contains some phytochemicals that may be protective or harmful to organs. For libron herbal supplement, some of its components such as cyclohexanol-1-ol, cyclohexane and Apoil were harmful to some organs as seen in an old study by Dixit *et al.* (1980) in which he administered 25 mg/kg of cyclohexanol-1-ol to rabbits for 40 days. It was seen to inhibit the process of spermatogenesis at the spermatocyte and spermatid levels, it reduced sialic acid concentration but there was no effect on the liver.

Another study by Tyagi *et al.* (1979) reveals severe reduction of the testes and seminiferous tubules upon exposure to cyclohexanol. Seminiferous tubules and Leydig cell nuclei were seen to be shrunken, sialic acid concentration dropped as such testosterone was reduced but no effect on the thyroid, kidney and liver cells. Newer studies by Sarvnarinder *et al.* (2021) using bisphenol shows lower sperm concentration, sperm motility an extensive vacuolisation in the germinal epithelium, abnormal basement membrane, and reduced germ cell in the positive control group.

Mascum herbal pride is seen to contain benzene, cyclohexasiloxane, cycycloheptasiloxane, diisocytyl and thioglycolic acid in the GC/MS report. Studies by Micheal *et al.* (1999) shows that siloxane compound is harmful to the liver whereas benzene was discovered to be harmful in studies by Aksoy, (2017). Similar reports were seen by Fayed *et al*. (2017) as he discovered significant increase in liver enzymes. Diisoocytyl phthalate was seen to act as endocrine disruptors and alters liver functions in a study by Manisha & Ravinder, (2016).

5. Conclusion

All the three herbal supplements assayed contain diverse phytochemicals that potrays its ability in handling male fertility but is also seen to posses some chemical compounds that may add up in altering the normal function of some biochemical system in accumulated quantity.

6. Recommendation

Further studies on the effect of these chemicals on various organs should be carried out.

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