A practical guide for transparent reporting of research on natural products in the *British Journal of Pharmacology:* reproducibility of natural product research

Introduction

Natural products continue to be an important source of medicines and drug templates. This is likely due to the vast chemical diversity of natural products, which increases the probability of finding structurally distinct "'lead compounds'" for different targets and diseases (Gu et al., 2013). This diversity is, in part, reflected by the size of natural product libraries that have been created, such as Supernatural II (http://bioinf-applied.charite.de/supernatural_new/index.php?site=home), which has 325,508 different natural compounds (Banerjee et al., 2015).

It has been estimated that approximately a third of all FDA-approved drugs over the past 20 years are based on natural products or their derivatives (Thomford et al., 2018). Considering the number of natural compounds that may be available for research, the potential for discovery of novel biological activity that might be relevant for human or veterinary therapeutics is substantial. For example, the Dictionary of Natural Products has thus far recorded ~200,000 plant secondary metabolites, including about 170,000 unique structures (Harvey et al., 2015) and the open access initiative (led by the U.S. National Cancer Institute) with libraries of ~80,000 plants, ~20,000 marine samples and ~ 6,000 microbes, estimates that ~1,000,000 distinct fractions will be derived over the next 3-4 years (https://dtp.cancer.gov/organization/npb/npnpd_prefractionated_library.htm).

In addition to the well-established role in drug discovery, natural product pharmacology is relevant in the context of the use of dietary supplements, which are part of the vast and nebulous nutraceutical market and includes vitamins, amino acids, proteins, minerals, fibres, plant extracts and natural compounds (Andrew and Izzo, 2017). Due to a lack of rigorous regulation in many countries, dietary supplements can be marketed without clinical evidence of efficacy and with uncertain composition (Andrew and Izzo, 2017). Despite this shortcoming, demand for dietary supplements continues to grow (Williamson et al., 2020). Herbal dietary supplement sales in the United States experienced record growth in 2018 and consumers spent $8.842 billion on herbal supplements across all market channels (Smith et al., 2018). Furthermore, traditional medicine (such as Ayurveda, Traditional Chinese Medicine, Unani, and many others) not only continue to form an integral part of treatment within certain cultures but have also entered Western culture (Williamson et al., 2020).

Considering the above, it is not surprising that BJP receives many submissions that focus on the pharmacology of specific natural products. For the great majority of natural product papers published in BJP, the Authors include final summative statements that propose a naturally-occurring molecule for further clinical development or immediate use as a dietary supplement in the nutraceutical market. Many of these papers also identify novel drug targets, elucidate the mode of action, discover lead compounds as well as offering potential natural product repositioning (see **Table 1** for a non-exhaustive list). All of these issues are highly relevant for BJP and offer novel pharmacological approaches to discovery science as well as potential therapeutics.

However, the acceptance rate of "'natural product'" submissions in BJP is lower than for manuscripts related to other topics (approximately 11% vs 22% acceptance rate). The editors of BJP consider natural product research as a fundamental field of pharmacology and seek to publish research in this area of the highest quality and with excellent reproducibility. In order to support

authors in understanding the expectations for natural product research manuscripts, we (the Senior Editorial team) have written this Editorial with the aims of i) providing clear advice to authors regarding the minimum standards required for publication in BJP so as to maximize the possibility of acceptance of such articles submitted to the Journal (and possibly elsewhere); ii) to help BJP Editors and reviewers in focusing on simple - but important – points that are often overlooked during the review process, but essential to support transparency and reproducibility of pharmacological natural product research.

1. BJP has a specific pharmacological focus

BJP does not publish papers that are limited to identification of natural products. Isolation, purification, elucidation of structure and semi-synthesis of chemical compounds are considered only in a context in which a robust, deep, and detailed pharmacological analysis and mechanism for biological activity attributed to the natural product are provided. As an example, in a BJP paper published by Yin and colleagues in 2015 the authors provided evidence of the synthesis of a previously isolated metabolite from Danshen (*Salvia miltiorrhiza*), which was identified by using mass spectrometry and proton and carbon nuclear magnetic resonance spectra. This information was provided along with detailed pharmacological analysis, which revealed that the natural compound prevented isoprenaline-induced cardiac fibrosis through inhibiting a NOX2/ROS/p38 pathway (Yin et al., 2015). Identification of the possible mechanism of action of the natural product is mandatory for publication in BJP.

2. BJP will publish studies on mixtures of compounds (such as herbal extracts) but only when accompanied by identification of the active component

BJP only considers papers that describe studies on purified active compounds; in such cases, the purity of the compound, as well as major impurities, must be reported. BJP does not consider papers on mixtures of compounds (such as herbal extracts alone) unless the manuscript also demonstrates that any activity evidenced is reproduced by a purified component of that extract. In some cases, the activity of an extract or mixture may be due to additive or synergistic activities of multiple compounds. If this is the case, then this finding should be shown by combining purified constituents and demonstrating functional activity that replicates the mixture. Overall, it is important to define which and how compound(s) of the extract is (are) contributing to the pharmacological activity.

As an example in which evaluation of an extract was followed by a detailed pharmacological analysis of the main active ingredient, a BJP paper reported that a number of distinct Chinese herbal medicine extracts increased the expression of uncoupling protein 1 (UCP1) in isolated adipocytes (Nie et al., 2018). The authors found that extracts from the Chinese plant *Astragalus membranaceous* had the greatest activity in this respect, then isolated and purified the components of that extract (with data from HPLC identifying the constituents) and identified the isoflavone formononetin as the chemical component responsible for the functional activity. Subsequently, the authors elucidated the pharmacological profile and the mode of action of formononetin, using a number of pharmacological and molecular approaches, and showed that it binds PPARγ, reduces weight gain and increases energy expenditure in obese mice.

However, the following are some exceptions in which BJP may consider studies on herbal extracts whereby discovery of the active components as potential therapeutics is not the aim of the study:

i) BJP will consider studies on chemically-characterized herbal extracts if a considerable clinical interest exists. An example is the case of the *Cannabis*-derived medicines, which are used for the treatment of spasticity associated with multiple sclerosis and for the treatment of paediatric

epilepsies. Whalley and colleagues evaluated the effect of diverse *Cannabis* extracts, with different concentrations of Δ^9-tetrahydrocannabinol (euphoric, convulsant) and cannabidiol (non-euphoric, anticonvulsant), on experimental seizures in animals (Whalley et al., 2019). The authors discovered that the controversy surrounding the reported proconvulsant effects of Δ^9-tetrahydrocannabinol was, in part, fuelled by major species differences in cannabinoid signalling and that these differences should be taken into consideration when seeking to better understand this potentially serious side effect.

ii) BJP will consider studies aimed at identifying the pharmacokinetic profile of single constituents after the administration of chemical-characterized, clinically well-studied extracts. For example, Chen and colleagues delineated the pharmacokinetics of flavonols and terpene lactones after dosing standardized *Ginkgo biloba* leaf extracts to rats via systemic or oral administration (Chen et al., 2013).

3. BJP requires full disclosure about extract and natural product preparation

Details regarding extracts must be provided: the part of the plant/marine entity or microbe used, the method of extraction, the yield of dried extract as a percentage weight of the starting fresh or dried material as well as type and concentration of extraction solvent. For all starting materials, the formal biological name should be given from which the extract is derived. The scientific name (including the family) should be used as per the Integrated Taxonomic Information System website and should be in the binomial format composed of the genus and species.

The source of the product (i.e. country and region) should be stated. For rare organisms and all plants a logged sample/voucher specimen stored within an accessible database in a recognised institution must be provided to enable others to access the sample and conduct analyses (Culley, 2013). Samples stored in personal and private stores and not available to other scientists are not considered acceptable

To ensure reproducible pharmacological activity, the extract must be chemically-characterized (e.g. by HPLC fingerprint, metabolomics) and the content of marker compound(s) measured with validated analytical methods. For extracted compounds, phytochemical characterization, purity (%) and methods used to determine compound identity and purity must be stated. Most compounds should be tested at high purity (95-99%). However, in some instances this may be difficult to achieve and if so, authors are required to provide a clear description of the other constituents and explain how these other components have been accounted for in the analyses. A figure that shows the structure of the extracted compound must be included. For commercially available compounds, the name of the supplier must also be included. Overall, reproducibility of findings is facilitated by the full disclosure of source of plant/tissue (provenance) and extraction process. To enable the latter, BJP has no word count restriction on methods. All extracts used must be available to the readership either through an open access facility or from the authors.

4. BJP requires that the effects of the vehicle employed must be tested and reported

Many compounds of natural origin are not easily soluble in water or saline and, hence, they may be dissolved in organic solvents, such as dimethyl sulphoxide (DMSO), ethanol, vegetable oils (sesame oil, corn oil) or ethanol. Unfortunately, many organic solvents, at relatively low concentrations, may effect cell lines or isolated tissue and interfere with outcomes of *in vivo* investigations. Cell-based assays are often intolerant to solvent concentrations of greater than 1% (Hughes et al., 2011). For example, DMSO, which is probably the most frequently used organic vehicle, exerts a number of pharmacological actions including differentiation of malignant cells, antioxidant and antibacterial activity, vasodilatation and smooth muscle relaxation as well as behavioural and *in vivo* cardiovascular effects (Jacob and Herschler, 1986; Castro et al., 1995; Parisi et al., 2010). Therefore, vehicles should be used at the lowest possible concentrations

ensuring solubilization and their effect on the responses under study must be clearly reported. Inadequate consideration of the effects of the vehicle can lead to incorrect judgement regarding intervention effects. A recent example of incomplete consideration of vehicle effects has occurred in the field of cardiovascular therapeutics with marine lipids. A substantial literature has reported positive effects of marine lipids upon circulating triglyceride levels; raised triglycerides correlates with worse outcome in terms of cardiovascular events (Sarwar et al., 2007). A large prospective study assessing the impact of icosapent ethyl in patients with raised triglyceride levels (REDUCE-IT) recently published its results (Bhatt et al., 2019). In this study the investigators assessed whether the refined marine lipid improved outcome for patients at risk. The results suggested that in comparison to the placebo, a mineral oil that mimicked the color and consistency of icosapent ethyl, icosapent ethyl reduced the cumulative incidence of cardiovascular events. However, for the subjects enrolled in REDUCE-IT, low-density lipoprotein cholesterol (LDL-C) was 75 mg/dL at baseline (on statins). In the active-intervention group, the LDL-C rose 2 mg/dL, whereas in the placebo (mineral oil) group it rose 7 mg/dL, for a net increase in LDL-C of 5 mg/dL in the latter. This level of LDL-C is known to be associated with an increase of events and thus raised the possibility that the icosapoent ethyl might be simply preventing the detrimental effects of another component of the fish oil.

There are also instances when in order to reach high concentrations of the compound under investigation (e.g., to construct a full concentration-response curve in agonist/antagonist studies), the vehicle can affect the response under study. In such cases, concentration-response curves for both the vehicle and the compound under investigation must be compared and shown. As an example, in BJP, Thomas and colleagues reported that the plant-derived compound cannabidiol displayed high potency as an antagonist of CB_1 and CB_2 receptors. The [^{35}S]GTPγS binding assay was used to determine both the efficacy of cannabidiol and the ability of cannabidiol to antagonize cannabinoid receptor agonists at the mouse CB_1 and the human CB_2 receptor. In this assay, the concentration-response curve related to the vehicle (DMSO) was clearly shown in figures, in comparison with the curve related to the effect of cannabidiol in the vehicle (Thomas et al., 2007)

5. BJP requires that a positive control is included

A further issue in the study of natural products is the need for testing a positive control, i.e. comparison of the natural compound under investigation with a well validated drug effective in the selected pharmacological model. Unfortunately, in many published papers, this requirement is ignored. Virtuous examples in BJP are below:

i) dexamethasone (s.c. treatment) and budesonide (aerosol treatment) were used as positive controls in evaluating the effect of the sesquiterpene α-humulene in an experimental model of airways allergic inflammation (Rogerio el., 2019).
ii) tirofiban (an antiplatelet drug belonging to the class of glycoprotein IIb/IIIα antagonists) was used as a positive control in investigating the protective effect of the anti-thrombotic agent anfibatide (a glycoprotein Ib antagonist derived from snake venom) in a murine model of brain ischaemia (Li et al., 2015).

The comparison should also consider the size of the effect and the possible clinical relevance. There are cases in the literature in which, although a conventional statistical significance is reached, the size of the effect is insignificant in the context of the disease target. As highlighted in a previous BJP editorial, a “10% change may be very relevant in some gene expression studies, whereas a 90% reduction in virus titre may be irrelevant in some viral infection studies” (Curtis et al., 2015).

6 BJP requires that the concentration used *in vitro* is appropriate for further pharmaceutical development

Cells in culture or isolated organs are often used in pharmacological research to screen new compounds isolated from natural sources and to delineate their mode of action. A major advantage of *in vitro* studies is that it is possible to test a broad range of high drug concentrations that cannot be reached *in vivo*. Whilst we do not prohibit the assessment of **only** high concentrations of active compounds in *in vitro* studies with few possibilities of *in vivo* application or inappropriate for further pharmaceutical development and drug discovery these are discouraged (Heinrich et al., 2020). There are many examples in the natural product literature in which high concentrations have been used to evoke a pharmacological effect that is then associated with claims for a therapeutic use (Gertsch, 2009; Butterweck and Nahrstedt, 2012). It has been argued that the molecular structures of natural compounds are such that they facilitate interaction with proteins and hence, at high concentrations, natural compounds are likely to produce unwanted effects (Gertsch, 2009). Indeed, testing compounds targeting specific proteins (e.g. receptors, enzymes) at concentrations that exceed by 10-fold the IC_{50} or Ki for the molecular target increases the possibility of introducing off-target actions (Smith and Houghton, 2013; Liston and Davis, 2017).

Unfortunately, there are no accepted limits beyond the cut-off concentrations commonly used in the pharmaceutical industry ($EC_{50} < 10$ μM) (Gertsch, 2009). Compound screening assays for hit discovery are typically run at 1-10 μM compound concentration (Hughes et al., 2011). A recent guide authored by editors of journals specialized in natural products research suggests that concentrations higher than 30-50 μM should not be used (Heinrich et al., 2020). BJP does not have a policy restricting concentration other than that the concentrations tested are achievable *in vivo* without causing unwanted biological effects (off or on target). For the evaluation of certain pharmacological activities, some suggestions are reported elsewhere. For example, antimicrobial activity is believed to be meaningful at concentrations below 25 μM (Cos et al., 2006). For antiproliferative/cytotoxic studies, it is recommended that compounds have selectivity and are not "anti-life" (Heinrich et al., 2020). In such studies, a comparison between the effect on tumors *vs* healthy cells is recommended. If the aim of the study is to identify the constituent responsible for effects of diet, the effects of the compound must occur at concentrations commensurate with those achieved from dietary consumption.

In general, it may be wise to ask the following question: "*can the concentrations used in vitro be present in the blood and tissue/cellular target after the administration of a therapeutic dose of the compound under investigation*"? The answer to this simple question requires knowledge of the pharmacokinetic profile of the compound under investigation.

An interesting study that investigated the *in vivo* relevance of a concentration used *in vitro* was recently published in BJP (Yeo et al., 2017). The authors found that the dietary polyphenol isorhapontigenin inhibited interleukin-6 (IL-6) release from airway epithelial cells. On the basis of pharmacokinetic data obtained in rats *in vivo*, the authors postulated that the concentration used *in vitro* can be reached following oral dosing of isorhapontigenin and that at least 30% inhibition of IL‐6 release can be attained through a single oral dose of isorhapontigenin (Yeo et al., 2017).

It is important to note that the compound under investigation might be a pro-drug - or, more generally, may be metabolized *in vivo* before the absorption phase and, hence, the results obtained *in vitro* could be misleading. This is common for natural products since many plant compounds exist in nature as glycosides, which are generally deglycosylated by intestinal microbiota, making the non-sugar portion available to be absorbed.

7. BJP requires that the dose used *in vivo* is translationally relevant

Extrapolation of dose from animal experiments to the human situation may be a difficult task (Nair and Jacob, 2016). In addition to body weight, a number of variables should be considered, including body surface area, pharmacokinetic parameters (clearance, volume of distribution) and inter-species differences in the pharmacodynamics. A comprehensive analysis of the principles of interspecies dose extrapolation is beyond the scope of this Editorial. Readers can refer to a paper published some years ago in BJP (Sharma and McNeill, 2009).

Allometric scaling of drug doses from preclinical experiments has been frequently used to predict the dose for single dose studies for first-in-human trials. This empirical approach is based not only on body weight, but on the normalization of dose to body surface area (mg/m^2). Tables created from FDA guidelines, available elsewhere (Sharma and McNeill, 2009; Nair and Jacob, 2016), represent a practical tool for the conversion of doses between animals and humans.

Unfortunately, interspecies dose conversion is very rarely considered. By analyzing BJP papers on natural products published in recent years, dose extrapolation is required in three main situations:

i) to promote the compound under investigation as a candidate for clinical development or as a dietary supplement (extrapolation from animals to humans).
ii) to investigate the mechanism of action of natural drugs or already marketed dietary supplements (extrapolation from humans to animals).
iii) to unravel the possible beneficial (or detrimental) effect of a natural compound in the context of a diet or an ingested food.

An example in which the dose has been correctly extrapolated from humans to animals can be found in BJP (Simeoli et al., 2017). The authors compared the effect of the dietary supplement butyrate (a postbiotic compound) with a more palatable butyrate-releasing derivative in a murine model of colitis. The daily dose (20 mg/kg) used in the mouse is convertible to a human equivalent dose (Nair and Jacob, 2016) of 113.4 mg, for an adult human subject weighing 70 kg. This dose is in the range or even less than the common dose of butyrate used in humans.

Conversely, one of the examples where dose ranges of activity do not match dietary approaches has been given by resveratrol, which, in an article published in *Nature* some years ago, was found to improve survival of mice on a high-calorie diet (Baur et al., 2006). By extrapolating the dose used in animals by using allometric scaling (Nair and Jacob, 2016), which considers body weight and surface area, the results suggest that a person would have to drink 55 bottles of wine per day.

In summary, dose ranges in animal experiments must be relevant from a preventive or therapeutic viewpoint and evidence supporting this must be provided. In general, multiple doses are generally recommended (in the context of compliance with the 3Rs—need to spelled out for readers). Single doses are accepted in complex pharmacological models only.

8. BJP requires that the route and timing of administration are appropriate

The route of administration plays a fundamental role in experimental design using animals. In traditional medical systems, most herbal remedies are administered orally, for example in the form of infusions or decoctions (Abdul and Huang, 2015). Similarly, natural products available in the market are generally dispensed in pharmaceutical forms for oral use. Therefore, the oral route of administration is preferred in pharmacological translational experiments related to natural products, especially when the study aims:

(i) to promote the compound under investigation as a dietary supplement;
(ii) to explain the possible beneficial effect of an ingredient found in the diet; or
(iii) to validate the traditional use of a natural compound (ethnopharmacological studies).

Thus, parenteral administration, such as s.c., i.p. or i.v. has little value in the context of the dietary supplements market and it should be limited only for studies related to conventional drug discovery.

In addition to traditional oral/parenteral dosing methods, compounds under investigation can be incorporated into the diet, as for example in a study published in BJP that assessed the impact of dietary supplementation for 2 months with the plant product quinic acid on glucose metabolism (Heikkilä et al., 2018). In order to determine how much compound needs to be mixed into the diet, it is essential to know key pieces of information, including the daily dose that the researcher aims to give to animals, the weight and usual daily food intake of the animal (Ricci, 2012). The inclusion of compounds into the diet has a precise significance when the study aims to unravel the relevance of a specific natural compound in the context of a diet.

The timing of drug administration (preventive *vs* curative) is also important. Compounds under study should not be given before administration of an agent used to induce an experimental disease, unless the authors are evaluating a possible candidate for prevention. For settings in which the goal is to cure rather than to prevent, compounds should be given after administration of the insult.

9. BJP requires that the potential toxicity of the compound under evaluation is considered

Many drugs fail in the clinic because they are not safe (Hughes et al., 2011). Thus, at the preclinical level, the determination of safety is as important as the proof of efficacy, both for submission to clinical trial as well as for promoting a natural compound as a food supplement. As a pharmacological journal, we do not ask for *in vivo* toxicology, but, whenever possible, authors are encouraged to provide information on the toxicity of the product under evaluation. For example, if a study is solely performed *in vitro*, some assessments of *in vitro* toxicity, such as cell viability or mitochondrial function are advised. For *in vivo* studies, measures of potential toxicity could include assessment, for example, of liver, renal or cardiac function. For selected pharmacological activities, such as antiproliferative/cytotoxic activity, the authors should show that extracts or compounds have selectivity *vs* cancer cells and are not “anti-life” drugs (Heinrich et al., 2020). Ideally, a comparison of the effect between cancer and healthy cells (if available), especially when the effect is observed at high concentrations, should be provided.
Finally, authors are also encouraged to review the literature to verify if toxicological information has already been reported. For example, Romano and colleagues found that the plant cannabinoid cannabichromene ameliorated experimental colitis at a dose which was more than 100-fold lower than the subacute LD_{50} dose reported in the literature (Romano et al., 2013).

10. BJP guidelines must be adhered to

Since 2016 the BJP has adopted a series of guidelines to support transparency and reproducibility. A major initiative has been the publication and implementation of guidelines related to design and analysis of experiments. The journal has published two editorials on this topic describing the requirements for submissions to the journal (Curtis et al., 2015; Curtis et al, 2018). Key issues highlighted in these guidelines include a need for blinding and randomisation in design, ideally evidence of sample size determination and a requirement for minimum n values of 5 prior to subjecting datasets to comparative statistical analysis. For all studies involving animals, animal tissues or primary cultures, authors must address issues raised in the BJP editorial “Implementing guidelines on reporting research using animals (ARRIVE etc.): new requirements for publication in BJP (McGrath and Lilley, 2015). Authors are encouraged to read BJP editorials on data presentation and sharing (George et al., 2017; George et al., 2019) as well as on the goals and the practicalities of immunoblotting and immunohistochemistry (Alexander et al., 2018). Checklists covering these

requirements are available on BJP website (https://bpspubs.onlinelibrary.wiley.com/hub/journal/14765381/declaration_english)

Finally, BJP now requires sex to be considered as a variable for all experimental reporting (Docherty et al. 2018). BJP editors recommend that all experiments (*in vitro, in vivo*, and *ex vivo*) should include both sexes, unless there is a specific justification not to do this.

Conclusions

This Editorial illustrates major requirements that authors should consider before submitting articles to BJP (see Table 2 for Authors checklist). It also highlights some common shortcomings in natural product pharmacological research, which could be prevented if experiments have appropriate planning and experimental design.

BJP is a leading journal in the pharmacological field, in which important new advances are published and thus novelty is a major determinant of acceptance for publication. Studies showing the effect of natural products, without a substantial investigation into the mode of action, are not considered. Articles limited to repetition of well-known data or that report similar pharmacological activities of similar chemical compounds are generally not suitable for BJP.

The editors of BJP recognise the wealth of opportunity for therapeutics that comes from the natural world and are keen to publish excellent natural product pharmacology that advances understanding of mechanisms both of physiological and pathological processes or that identifies potential new therapeutics.

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Table 1. Examples of articles on natural products published in the BJP

Article type	**Example in the BJP**	**Reference**
Papers promoting a phytochemical for possible clinical investigation	Betulic acid, via FXR activation, attenuated non-alcoholic fatty liver disease (NAFLD) in an animal model of hepatic steatosis. The Authors conclude that "Our data suggest that the effects of Betullic acid may be used to develop a novel therapy for the management of NAFLD"	Gu et al., 2018
Papers promoting a phytochemical for immediate use in humans as a dietary supplement	β-Caryophyllene protects against experimental alcoholic steatohepatitis The Authors conclude that "Our study may have immediate translational potential in liver disease as BCP is an FDA approved food additive for humans"	Varga et al., 2018; Br J Pharmacol 175 320–334
Papers investigating the effect of a phytochemical in the context of a diet	Resveratrol exists in high quantities in certain foods such as muscadine grape and red wine (Durazzo et al., 2019). A BJP paper found that resveratrol, incorporated into the diet, at a dose able to achieve relevant circulating resveratrol levels, protected against bone loss.	Zhao et al., 2012
Paper providing the pharmacological basis able to justify the use of a dietary supplement in humans	Palmitoylethanolamide (PEA) is a food supplement marketed to alleviate inflammatory bowel disease, even in absence of clinical trials substantiating this claim. A BJP paper found that oral administration of PEA is therapeutic in a murine model of colitis. The Authors believe that the results could justify the use of PEA for IBD in humans This example is reminiscent of the ethnopharmacological approach, i.e. the study of natural medicines that have been traditionally used by ethnic groups. Ethnopharmacology uses the "reverse pharmacology approach", which was applied in India to develop medicines from Ayurvedic medicines (Patwardhan and Mashelkar, 2009)	Borrelli et al., 2015
Papers depicting the mode of action of a commercially-available dietary supplements	PEA is a food supplement with a well-stablished analgesic activity in animals. A BJP paper unveiled the molecular mechanism involved in PEA-induced analgesic effects by showing its ability to activate and desensitize TRPV1 in sensory neurons	Ambrosino et al., 2013
Papers depicting the mode of action of a major ingredient contained in a commercially-available dietary supplement	Extract from *Petasites hybridus* (butterbur) are used for migraine prevention. A BJP paper showed that a isopetasin, a major ingredient of butterbur, activated TRPA1 channels, resulting in excitation of neuropeptide-containing nociceptors, followed by marked heterologous neuronal desensitization. The Authors conclude that "such attenuation in pain and neurogenic inflammation [by isopetasin] may account for the anti-migraine action of butterbur".	Benemei et al., 2017

Papers repositioning old natural drugs	Digoxin inhibited endothelial focal adhesion kinase and angiogenesis induced by different growth factors. The Authors conclude that "These novel findings suggest a potential repositioning of digitoxin as a broad-spectrum anti-angiogenic drug for diseases where pathological angiogenesis is involved"	Trenti et al., 2017
Papers related to naturally-occurring drugs of abuse	A BJP paper investigated the involvement of the CRF2 receptor in social dysfunction and stress vulnerability induced by repeated administration of cocaine and withdrawal from cocaine. The Authors conclude that "These findings demonstrate a central role for the CRF2 receptor in social behaviour deficits and biomarkers of vulnerability induced by cocaine withdrawal"	Morisot et al., 2018
Papers promoting a phytochemical as lead compound for the development of new medicines	Novel tetracyclic triterpenoid compounds, isolated from the mushroom *Poria cocos*, were demonstrated to be effective against experimental renal fibrosis. The Author conclude that "Our results provide several potential leads for the development of novel compounds for effective treatment of chronic kidney disease"	Wang et al., 2018
Papers in which a natural compound is used as a chemical probe to block or activate a specific target	Acid-sensing ion channels (ASICs) are voltage-insensitive cation widely expressed in central and peripheral nerves. Peptide toxins from animal venoms, such as mambalgins, target different ASIC subtypes. In a BJP paper, mambalgin-1, a specific inhibitor of ASIC1a- and ASIC1b-containing channels, was used as a chemical probe to reveal the role of such channels in mechanical allodynia in a rodent model of migraine	Verkest et al., 2018
Papers comparing the effect of a natural compound with a related semi-synthetic compound	Dihydrodiosgenin, the parent aglycone of diosgenyl saponin, protected against pancreatic acinar cell against three clinically relevant models of experimental acute pancreatitis	Shen et al. 2018
Papers reporting the effect of a naturally-occurring molecule on specific targets (e.g. receptors, enzymes)	The diterpene ester tonantzitlolone displayed nanomolar potency as an activator of transient receptor potential canonical (TRPC) 1/4/5 channels	Rubaiy et al., 2018
Papers providing pharmacokinetic data	*Ginkgo biloba* extracts are widely promoted for conditions related to microcirculatory and memory deficits. A BJP paper delineated the pharmacokinetics of flavonols and terpene lactones (i.e. the main active ginkgo ingredients) after dosing standardized *Ginkgo biloba* leaf extracts to rats.	Chen et al., 2013

Papers promoting a natural compound for cosmetic use	Kazinol U is a prenylated flavan isolated from the Chinese and Japanese plant *Broussonetia kazinoki*. A BJP paper showed that kazinol U reduced melanogenesis. The Authors conclude that "These findings indicate that kazinol U might be therapeutically and cosmetically applied for several hyperpigmentation skin disorders and skin whitening"	Lim et al
Papers describing novel formulations for dietary supplement delivery	Curcumin is a widespread dietary supplement whose clinical use is limited by the poor oral bioavailability. A BJP paper provided evidence that biodegradable nanosystems encapsulating have the potential to better translate to humans	Ganugula et al., 2017

Table 2. Authors checklist for manuscripts to be submitted to the British Journal of Pharmacology

	Questions	**Comment/Advice**
1	Is the mechanism of action of the natural product reported?	BJP seeks to advance understandings of the mechanisms of action of pharmacological compounds
2	Does the study report the activity of a mixture of compounds (e.g. herbal extracts) or a pure compound?	BJP ordinarily does not publish papers on mixture of compounds unless the manuscript also demonstrates that any activity evidenced is reproduced by a purified component of that mixture. Purity must be evidenced and % stated
3	What is the origin of the natural product?	Methods must disclose source, extraction and purification/synthesis process
4	Has the effect of the vehicle on the response under study been reported?	Vehicle effects must be clearly reported, with mean ± SEM or SD?, appropriate statistical analysis and with $n \geq 5$.
5	Has a positive control been used?	The effect of the compound under investigation should be compared with a clinically-effective drug. If the positive control has been not reported, include a valid scientific justification (e.g. there are no clinically-effective drugs for the specific disease). Discuss the size of the effect in relation to the disease under evaluation.
6	Are the concentrations used *in vitro* appropriate for further pharmaceutical development?	Rationale for the selection of concentrations used must be provided. Include a valid scientific justification if high concentrations (e.g. ≥25 μM) are used. Pharmacokinetic data on plasma levels following systemic administration should be reported. Concentrations much higher than the IC_{50} or Ki reported for the compound under investigation must not be used.
7	Are the doses used *in vivo* relevant for translation?	The rationale for the selection of the doses used must be provided. If testing as a potential therapeutic, relevance to the clinical setting must be provided.
8	Is the dosing schedule appropriate?	The rational for the selection of the route, timing and frequency of administration should be provided. to cure rather than to prevent (or *vice versa*).
9	Is the compound under investigation safe?	Evidence of safety should be provided, or if not available, discuss issues regarding potential toxicity.
10	Are experimental design and analysis, data presentation and sharing in line with BJP guidelines? Has been sex considered as a biological variable?	BJP guidelines must be adhered to, using Editorials as a reference (Curtis et al., 2018; George et al., 2017; George et al., 2019; Alexander et al., 2018; Docherty et al., 2019). Checklists are available on BJP website.